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Studies of the embryonic response to weak changes of the electromagnetic environment were performed, in vivo, on chick embryos. Fertile eggs were exposed to weak, extremely low frequencies, bipolar, pulsed magnetic fields during their first 48 hours of incubation. The development of the embryos was analyzed at the end of the exposure and incubation period. This analysis consisted of a double blind description of the morphological characteristics for five organogenetic systems: cephalic and truncal nervous systems, somites, heart and extraembryonary vessels. In each experiment, a control sample was simultaneously incubated, but not exposed to the EMF and analyzed together with the experimental group. The effects of the exposure were estimated comparing the proportions of normal and not normal embryos in the two groups.
The different studies have shown that weak, ELF magnetic fields can induce an increased incidence of abnormal development. (1) The effects of the fields lead to the embryonic death, occurring during the first weak after the exposure, or to malformations in alive organisms. (2) The

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developmental stage of the embryos at the starting time of the EMF exposure seems to be determinant in the embryonic response. The organisms are sensitive to a 20 hours field exposure started during the second day of their development, at stages 9+ -10. (3) The electric fields induced by time varying magnetic fields intervene in the biological effects. (4) The sensitivity of the embryonic organisms to a PMF also depends on their orientation. (5) A pulsed magnetic field exposure can modify the orientation of the embryos. (6) The orientations of embryos from different strains and breeds show different distributions. Two breeds respond differently to a same PMF exposure. (7) PMFs effects could be modulated by slight changes of the local geomagnetic field (GMF). The end of the gastrulation was shown to be the most sensitive stage to the PMFs incidence, in relation to the magnitude or the inclination angle of the GMF. (8) The embryonic development in "control" conditions seems to be dependent on the GMF, especially at the end of the gastrulation phase. This relationship is not season dependent. (9) A 30 Hz PMF can stop the embryonic development before the begining of the neurulation phase. This AC field frequency was calculated, taking into account that the ambient DC field had a 44.2 uT magnetic flux density, for a possible resonant effect on sodium and calcium ions transport. Even though all the conditions for a maximum cyclotronic resonance of these ions were not satisfied, the biological response supported the hypothesis of their alteration. (10) An increased incidence of developmental abnormalities was observed on embryos exposed to a combination of AC and DC magnetic fields, determined according to the model of cyclotronic resonance for calcium ions. However, the effect was observed only on the truncal nervous system and the somites. This preliminary study suggests that the cyclotronic resonance model could explain, in part, the effects of time-varying MFs on developing organisms. (11) The results of our experiments included in the Henhouse project have shown that the unipolar PMF used in this study, did not change significantly the proportions of normal and not normal embryos. However, those with strong malformations were significantly increased whereas those with slight anomalies decreased. This result suggests that embryos with different physiological states (normals or not normals) can respond differently to a weak PMF exposure.

# STUDIES OF WEAK, ELF ELECTROMAGNETIC FIELDS EFFECTS ON THE EARLY EMBRYONIC DEVELOPMENT

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# GENERAL INTRODUCTION

Our purpose is the study of the parameters and conditions that influence the effects of weak ELF magnetic fields on the early embryonic development.

In previous studies, we observed that the early chick embryo is sensitive to weak ELF pulsed magnetic fields (PMFs): A 48 hour exposure, in vivo, can induce developmental anomalies in the organism. This effect is associated to abnormal figures of the glycosaminoglycan components of the embryonic tissues and to a loss of cellular adhesiveness and cohesion.

This effect was found to be dependent on the waveform, the pulse repetition rate and the flux density of the field as well as on the orientation of the embryo (Delgado et al., 1982; Ubeda et al, 1983, 1987).

We also observed that a five hour exposure of organisms in stage 7 (30 hours of incubation) or stage 9 (38 hours of incubation) is sufficient to produce changes of the glycosaminoglycans and the mitotic index of the embryonic neural tissue.

From June 1985 to the present date, the studies were performed in two different ways. The first one was the continuation of our previous work, trying to know (1) the long-term effects of different PMFs exposures, occurred during the first two days of the embryonic development (post laying); (2) the biological responses to a field exposure started at different developmental stages; (3) the possible role of the electric field (EF) induced by the magnetic field; (4) the responses of the

embryos in relation to the orientations of the organisms and to the orientations of the MFs; (5) the possible influence of slight changes of the local DC field on the PMFs effects and the embryonic development; (6) the effects of a 30 Hz PMF. (7) The response of the organisms exposed to a combination of AC and DC fields, determined according to the model of cyclotronic resonance for calcium ions.

The second aim of our work was the preparation, the performance and interpretation of a series of experiments done, in collaboration with five other laboratories, on the effects of a weak, ELF pulsed magnetic field on chick embryos (Henhouse Project). The purpose of this collaborative program was to study the field effects on embryos incubated and exposed in the same controlled conditions in the six laboratories, so that the results could be compared and grouped together.

## MATERIAL AND GENERAL METHODOLOGY

The biological system was White Leghorn chick embryos.

All the experiments were performed in vivo, i.e, on fertil eggs, provided by the same local farm, from hens at the same stage of maturity (between 7 and 13 months) and whose food was strictly controlled, (not changed) by the veterinary of the farm in collaboration with us.

The eggs were provided to the laboratory within 12 hours after laying. They were stored at 10-15° C, always at the same place, horizontally, with the narrow end pointing west. They were

used within three days after laying. Those whose shell presented any abnormal structure or aspect were excluded.

The eggs were randomly chosen, a part of them being incubated inside stimulated coils located in an incubator, while the other part was incubated inside a no stimulated coil (sham-exposed eggs) or without coil (controls), in another incubator.

This methodology, in particular the use of two incubators, one for the MF exposed eggs and another for the sham or controls was adoped for the following reasons.

1.- When coils are stimulated, field intensities reaching 0.4 to 2.2 % of the flux density induced in the coils are measured in different zones of the incubator. In some experimental conditions (for example with a 100 μT flux density) this "contamination" outside the coils reaches values known to be effective on the embryonic development and in other conditions it reaches values which effectivity on the embryons is not yet known.

2.- The MF exposed eggs were always in the same incubator and the sham or controls in the other one. Previous studies had shown that the embryonic development was similar in the two incubators (measured by the proportion of normal embryos in the samples and the mean stage reached by the organisms at the end of a same incubation time).

3.- Our parametrical studies as well as those on the field orientation etc. must be comparative each one with the others. Therefore, it was necessary to use always the same

incubator for all the MFs exposed eggs and the same other one for the controls or sham-exposed.

4.-In some series of experiments, the MF exposed samples were compared to control eggs and not to sham-exposed: The incubation inside no stimulated coils does not change the embryonic development when compared to controls incubated outside coils (Ubeda et al, 1983).

The eggs were incubated horizontally, their narrow end pointing west and not moved during the 48 hour exposure and incubation time.

In the incubators, the temperature was 38.0±0.2°C and the relative humidity 60±5%. In the case of the Henhouse project (HHP) experiments, the temperature of incubations was 37.8±0.3°C and the RH 75-83%.

Except when indicated, the MF was horizontal, east-west oriented. Current into the coils produced a bipolar pulse with a repetition rate of 100 Hz, a duration of 500 μsec and a 1 μT peak-to-peak magnetic flux density. In the case of the HHP experiments the pulse was unipolar.

At the end of the exposure and incubation time, the eggs were removed and the embryos double blind described. They were classified as normal or not normal. The **Not normal** embryos included the **non developed** (stage lower or equal to stage 4, which is the stage of the definitive primitive streak), the **abnormals** (embryos with a normal morphology according to their stage but which had only reached a stage between 4 and 9; or those with a stage higher than 9 but with a slight morphological

anomaly) and the malformed embryos (showing strong morphological anomalies).

In each experiment, the proportions of Not Normal embryos in the MF exposed sample and in the sham-exposed or control group were compared. At the end of a series of similar experiments the data were analyzed. Depending on the study performed, the statistical test used was the binomial test of percentage comparison (Snedecor and Cochran, 1967) or the analysis of variance.

## RESULTS OF THE DIFFERENT STUDIES

### 1.- LONG-TERM EFFECTS OF PMFS EXPOSURES OCCURRED DURING THE FIRST 48 HOURS OF DEVELOPMENT (POST LAYING)

- Introduction and Methods:

Our previous results had shown that chick embryos exposed, in vivo, during the first two days of incubation to weak PMFs with a 100 Hz pulse repetition rate and 1.0 μT flux density, exhibited, at this time, increased proportions of developmental abnormalities. The effects of the PMFs seemed to be dependent on the magnetic flux density and waveform (Ubeda et al, 1983).

The purpose of the present study was to know if the field effects, measured by the proportions of normal and not normal embryos at the end of the exposure, was a valid methodology to estimate the actual incidence of the PMFs on the organisms.

As shown in table 1, results of previous studies showed that three PMFs induced different abnormality ratios (ARs) at the end of the 48 hours. These experiments were repeated.

The three PMFs were applied to different samples of fertil eggs during the first two days of incubation (without moving the eggs) in similar conditions than those previously described (Ubeda et al, 1983). The control samples were incubated simultaneously but not exposed. The pulsed current was established in five cylindrical coils connected in series (field A) or in a Helmholtz coil (field B), using a Grass SD9, 200 Hz pulse generator. The magnetic flux densities were 1.0 and 104 μT

for field (A) and 1.0 μT for field (B) and the pulse rise times, 85, 100 and 2 μsec respectively. In the last case (field B) the pulse was rectangular with a 2 usec rise and fall times.

Instead of to open the eggs and analyse the embryos at the end of the two days of exposure and incubation, the field exposed and control eggs were removed and put to incubate in a poultry incubator (turning over the eggs every two hours), at 38±0.2°C and 65±5% RH, for 9 additional days.

In the process of transport of the eggs from the first incubator to the poultry one, the eggs were maintained at the laboratory temperature (22°C) during 1.5-2.0 minutes. A study done on the temperature inside the eggs during this process showed that the temperature decreased to 37.8°C and returned to 38°C in six minutes (Fig 1).

At the end of the 11th day of incubation, the eggs were removed and the embryos double blind described. The stage normally reached after a 11 day incubation at 38°C is stage 37, according to Hamburger and Hamilton (1951). In each embryo we studied the following characters:

- General aspect: stage; size of the embryo; size of the head relatively to the trunk.

- Head: morphology of the skull; development of the eyes; size and morphology of the beak; size of the neck.

- Trunk: morphology of the vertebral column; development of the tail; closure of thorax and abdomen.

- Limbs: size and morphology.

The organisms whose development had been stopped at an early phase presented a high degree of necrosis and were described with

many limitations.

An embryo was considered normal when its developmental stage was at least stage 36 and its morphollogy was normal. A not normal embryo was an organism with, at least, one morphological abnormality or whose stage was lower than stage 36.

**Results and Discussion:**

- Control *embryos*

A total number of 276 control embryos were incubated during 11 days in the conditions anteriorly described. Table 2 shows the cases of dead and/or malformed embryos, divided chronologically in two groups. In the first group were included the embryos whose development was stopped during the first week (31 among 276 i.e 11.2%). All of them were dead. In the second group were included the embryos developed up to, at least, day 8 of incubation. The results showed that the Not Normals represented a 12.0% of the population (Table 2). The majority of ths deaths had occurred during the first 5 days of incubation. According to Allcroft and Beer (1973), 20 % of the embryos do not reach hatching. It is also well known that for the chick embryo the cycle of development presents two periods in which the embryonic death is specially increased: between days 3 and 5 and between day 18 and 20 of incubation.

In this study, the opening of the eggs at day 11 did not allow a rigorous description of the embryos dead during the first three days of incubation (necrosis of the tissues). But for those

dead later, the abnormality more frequently observed affected the extraembyonic vascularization. Two embryos morphologically malformed had a small size and presented malformations of cephalic structures (microphtalmia and crossed peak).

**-Embryos exposed to PMFs:**

- Field A, with a 1 $\mu$T flux density (pulse rt= 85 $\mu$sec): Seventy eight exposed embryos were compared to 75 controls. The results (Table 3) showed that the field exposure did not induce embryonic death during the first week (AR = 1.1). However, the field induced an increased incidence of malformed embryos with a development corresponding to 10-11 days (AR = 6.4; p = 0.059). The malformations were localized in the peak, the eyes and the skull, and in the closure of the thorax and abdomen. The total of not normal organisms represented 8.0 % of the controls and 15.4 % of the field exposed embryos. The relative effect of the exposure, expressed as the abnormality ratio was 1.9 (p = 0.211). The field exposure during the same period (48 hours) induced, at the end of the exposure, a similar relative increase of abnormalities (AR = 1.8; p = 0.067; Table 1).

- Field A, with a 104 $\mu$T flux density (rt: 100 $\mu$sec): The results of this field exposure (Table 4) showed no significant change of the proportion of not normal organisms (27.1%), compared with the controls (18.9%; p = 0.381; AR = 1.4). The data seem to confirm the results obtained at the end of the two days exposure (Table 1). Nevertheless, the analysis also suggests that the field exposure could induce early deaths, between days 2 and 4, (11.9 % instead of 1.7 %; p = 0.061) and a slight

increase of not normal organisms whose abnormalities are expressed from the end of day 2 of exposure and incubation. More experiments are necessary to conclude.

- Field B, with a 1 μT flux density (rise and fall times 2 μsec): The embryos exposed to this PMF (N = 82) showed, after 11 days of incubation, two responses: Early deaths and malformations in embryos developed up to day 10 (Table 5). The proportion of dead organisms with a development lower than a day was significantly increased (11 % instead of 2.2% among 92 controls; p = 0.026). The sum of the dead during the first two days is 14 among the 82 exposed (17.1 %) and 5 among the 92 controls (5.4 %; p = 0.016; AR = 3.2). These results reinforce the data obtained at the end of the 48 hour exposure (Table 1) and suggest that the field induces early, lethal malformations of the cephalic nervous system, the trunk and the limbs. The field is clearly teratogenic on embryos developed up to day 10, inducing a 6 times increase of malformed, specially for cephalic structures. The total proportion of not normal embryos was 29.3% instead of 12.0% in the control sample (p = 0.005), which represent an AR of 2.5, similar to the AR found at the end of the 48 hours exposure time.

The results of these three series of experiments support the results previously obtained by the analysis of the exposed and control populations at the end of the 48 hours. The data obtained in the present study at day 11 of incubation, showed a similar or higher incidence of the fields than at day 2.

Therefore, the methodology of a 48 hour exposure and incubation with a morphological analysis of the embryos at this time, provides valid estimation of a field incidence on chick embryos.

## TABLE 1

Effects of field (A) with a 1.0 μT and 104 μT flux density and field (B) with a 1.0 μT flux density at the end of a 48 hour exposure. (AR = Abnormality Ratio i.e % Not Normals in Exposed group / % Not Normals in Controls) (*) data published in Ubeda et al, 1983.

| | A (*) | | | | B | |
|---|---|---|---|---|---|---|
| MF rise time | 85 μsec | | 100 μsec | | 2 μsec | |
| MF flux density (μT) | 1.0 | | 104 | | 1.0 | |
| | Controls | Exposed | Controls | Exposed | Controls | Exposed |
| Total Number of Embryos (N) | 55 | 55 | 53 | 59 | 39 | 50 |
| Normals | | | | | | |
| n | 42 | 32 | 40 | 42 | 34 | 34 |
| % | 76.3 | 58.1 | 75.4 | 71.1 | 87.2 | 68.0 |
| mean stage | 12.9±1.0 | 13.0±0.6 | 11.6±1.3 | 11.6±1.2 | 11.2±1.4 | 10.7±1.3 |
| Not Normals | | | | | | |
| n | 13 | 23 | 13 | 17 | 5 | 16 |
| % | 23.6 | 41.6 | 24.5 | 28.8 | 12.8 | 32.0* |
| AR | 1.8 | | 1.2 | | 2.5 | |
| (p) | 0.067 | | 0.672 | | 0.045 | |
| Non Developed | | | | | | |
| n | 2 | 2 | 4 | 3 | 0 | 5 |
| % | 3.6 | 3.6 | 7.5 | 5.0 | - | 10.0 |
| (p) | 1.000 | | 0.706 | | 0.065 | |

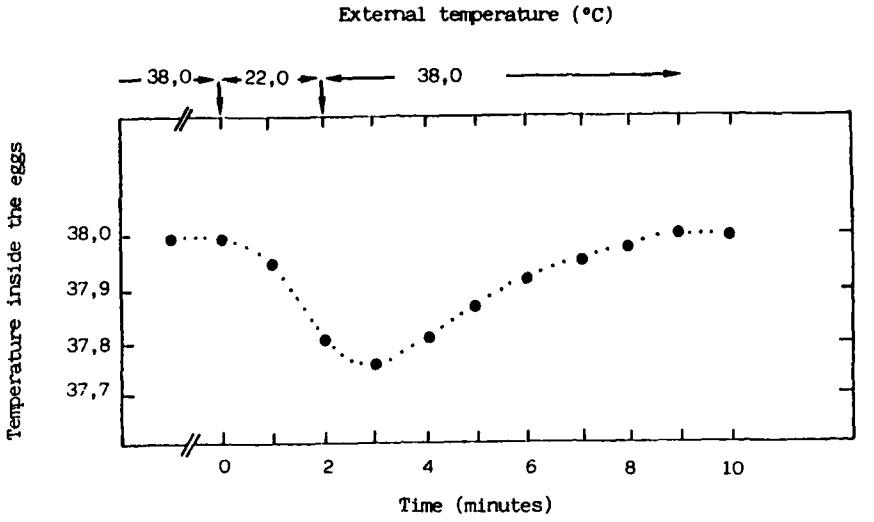


Fig. 1.- Changes of the temperature inside eggs before, during and after their transport to the poultry incubator.

## TABLE 2

Cases of dead and/or malformed organisms observed after a 11 day incubation, among 276 control embryos.

| | Number | % | Number of dead | Description |
|---|---|---|---|---|
| Development: 1st Group | | | | |
| - Lower than 1 day | 15 | 5.4 | 15 | Necrosed-Amorphous |
| - Two days | 4 | 1.4 | 4 | Necrosed-few vascularization in the blastoderm |
| - Three days | 2 | 0.7 | 2 | Necrosed-few vascularization in the blastoderm |
| - Four days | 5 | 1.8 | 5 | Necrosed-Malformed CNS and vascularization |
| - Five days | 5 | 1.8 | 5 | Necrosed-Normal morphology |
| Total 1st group: | 31 | 11.2 | 31 | |
| Development: 2nd Group | | | | |
| - 9-10 days | 2 | 0.7 | 0 | Small size of the embryos. Delay in the development. 1 case with malformed peak (0.3%) and the other case with microphtalmia unilateral (0.3%) |
| Total of the Two Groups (Dead and Malformed alive) | 33 | 12.0 | 31 | |

## TABLE 3

Cases of dead and/or malformed organisms observed after a 11 day incubation among 75 controls and 78 embryos exposed to field (A) 1 μT (rt: 85 μsec), during the first 48 hours of incubation.
C = Controls; E = Exposed; AR = Abnormality Ratio

| DEVELOPMENT | Number | % | Dead | Description |
|---|---|---|---|---|
| First group: | | | | |
| - Lower than 1 day | | | | |
| C | 4 | 5.3 | 4 | Necrosed. Amorphous. |
| E | 3 | 3.8 | 3 | Necrosed. Amorphous. |
| (p) | | NS | | |
| - Two days | | | | |
| C | 0 | - | 0 | |
| E | 3 | 3.8 | 3 | Necrosed. Amorphous. |
| (p) | | NS | | |
| - Four days | | | | |
| C | 2 | 2.6 | 2 | Necrosed. Malformed nervous system and vascularization (2). |
| E | 0 | - | 0 | |
| (p) | | NS | | |
| - Six days | | | | |
| C | 0 | - | 0 | |
| E | 1 | 1.2 | 1 | Necrosed. Malformed nervous system and vascularization. |
| (p) | | NS | | |
| - Total 1st group: | | | | |
| C | 6 | 8.0 | 6 | |
| E | 7 | 8.9 | 7 | |
| (p) | | NS | | |
| AR | | 1.1 | | |
| Second group: | | | | |
| - 10 days | | | | |
| C | 0 | - | 0 | |
| E | 1 | 1.2 | 1 | Normal Morphology. |
| (p) | | NS | | |
| - 11 days | | | | |
| C | 0 | - | 0 | |
| E | 4 | 5.1 | 0 | Malformed Skull (2); Anophthalmia: Unilateral (1) Bilateral (1) Crossed peak (1); Thoraco-gastroschisis (1) |
| - Total 2nd group: | | | | |
| C | 0 | - | 0 | |
| E | 5 | 6.4 | 1 | |
| (p) | | 0.059 | | |
| AR | | 6.4 | | |
| TOTAL | | | | |
| C | 6 | 8.0 | 6 | |
| E | 12 | 15.4 | 8 | |
| (p) | | 0.211 | | |
| AR | | 1.9 | | |

TABLE 4

Cases of dead and/or malformed organisms, observed after a 11 day incubation, among 58 controls and 59 embryos exposed to field (A) 104 μT (rt: 100 μsec), during the first 48 hours of incubation.
C = Controls; E = Exposed; AR = Abnormality Ratio

| DEVELOPMENT | | Number | % | Number of dead | Description |
|---|---|---|---|---|---|
| First group: | | | | | |
| - Lower than 1 day | | | | | |
| | C | 4 | 6.9 | 4 | Necrosed. Amorphous. |
| | E | 6 | 10.1 | 6 | Necrosed. Amorphous. |
| | (p) | | NS | | |
| - Two days | | | | | |
| | C | 1 | 1.7 | 1 | Necrosed. Amorphous. |
| | E | 5 | 8.4 | 5 | Necrosed. Amorphous. |
| | (p) | | NS | | |
| - Three days | | | | | |
| | C | 0 | - | 0 | |
| | E | 1 | 1.7 | 1 | Necrosed. Malformed vascularization. |
| | (p) | | NS | | |
| - Four days | | | | | |
| | C | 0 | - | 0 | |
| | E | 1 | 1.7 | 1 | Necrosed. Small size. Malformed vascularization. |
| | (p) | | NS | | |
| - Seven days | | | | | |
| | C | 5 | 8.6 | 5 | Normal morphology(5). |
| | E | 1 | 1.7 | 1 | Normal morphology. |
| | (p) | | NS | | |
| -Total 1st group: | | | | | |
| | C | 10 | 17.2 | 10 | |
| | E | 14 | 23.7 | 14 | |
| | (p) | | 0.493 | | |
| | AR | | 1.4 | | |
| Second group: | | | | | |
| - 10 days | | | | | |
| | C | 1 | 1.7 | 0 | Crossed peak. |
| | E | 2 | 3.3 | 0 | Anencephalia (2). |
| | (p) | | 1.000 | | |
| | AR | | 1.9 | | |
| TOTAL | | | | | |
| | C | 11 | 19.0 | 10 | |
| | E | 16 | 27.1 | 14 | |
| | (p) | | 0.381 | | |
| | AR | | 1.4 | | |

TABLE 5

Cases of dead and/or malformed organisms observed after a 11 day incubation among 92 controls and 82 embryos exposed to field (B) 1 pf (rt 2 μsec), during the first 48 hours of incubation. C = Controls; E = Exposed; AR = Abnormality Ratio.

| DEVELOPMENT | Number | % | Number of dead | Description |
|---|---|---|---|---|
| **First group:** | | | | |
| - Lower than 1 day | | | | |
| C | 2 | 2.2 | 2 | Necrosed. Amorphous. |
| E | 9 | 11.0* | 9 | Necrosed. Amorphous. |
| (p) | | 0.026 | | |
| - Two days | | | | |
| C | 3 | 3.2 | 3 | Necrosed. Malformed vascularization (3). |
| E | 5 | 6.1 | 5 | Necrosed. Malformed vascularization (5). |
| (p) | | NS | | |
| - Total for developed up to 2 days | | | | |
| C | 5 | 5.4 | 5 | |
| E | 14 | 17.1* | 14 | |
| (p) | | 0.016 | | |
| AR | | 3.2 | | |
| - Three days | | | | |
| C | 2 | 2.1 | 2 | Necrosed. Malformed Cephalic NS (2) and vascularization (2). |
| E | 3 | 3.6 | 3 | Necrosed. Small size (3). Malformed Cephalic NS (3) and vascularization (3). |
| (p) | | NS | | |
| - Four days | | | | |
| C | 3 | 3.2 | 3 | Necrosed. Small size (3). Malformed vascularization (3). |
| E | 1 | 1.2 | 1 | Necrosed. Malformed Cephalic NS, trunk, limbs and vascularization. |
| (p) | | NS | | |
| - Total 1st group: | | | | |
| C | 10 | 10.9 | 10 | |
| E | 18 | 22.0 | 18 | |
| (p) | | 0.062 | | |
| AR | | 2.0 | | |
| **Second group:** | | | | |
| - 10 days | | | | |
| C | 1 | 1.1 | 0 | Microphthalmia Unilateral. |
| E | 6 | 7.3 | | Anophthalmia (2); microphthalmia (1); small size (3); malformed skull (3), peak (2), neck (1); short trunk (1); thoraco-gastroschisis (3). |
| (p) | | 0.053 | | |
| AR | | 6.6 | | |
| TOTAL | | | | |
| C | 11 | 12.0 | | |
| E | 24 | 29.3** | | |
| (p) | | 0.005 | | |
| AR | | 2.5 | | |

## 2.-EMBRYONIC RESPONSE TO A FIELD EXPOSURE STARTED DURING THE PRIMARY NEURULATION PHASE

### Introduction and methods

The purpose of this study was to know if the embryos are sensitive to a weak, ELF pulsed magnetic field, the exposure being started, during the early processes of the primary neurulation.

For that aim, it was used a pulsed magnetic field which had a slight effect on the embryos, when applied from the begining of the incubation up to 48 hours. Its characteristics were the following: bipolar pulse (average zero) with a 100 Hz repetition rate, 1 $\mu$T peak-to-peak amplitude, 500 $\mu$sec. duration and 85 $\mu$sec. rise time (pulse A in Table 1, page 12).

The methodology used in the present study was the following: Fertil eggs were incubated during 38 hours. At this time, they were removed from the incubator and the embryos examined "in situ", through a small window opened in the middle upper part of the shell. The gross examination of the embryos (using a Nikon binocular stereomicroscope) was done to select those at stages $9^{+}$-10 with a normal morphology. The windows in the shells were then closed (using steril pieces of shell) and the selected eggs returned to incubate for 20 hours, inside stimulated coils (exposed embryos) or outside coils (controls). Therefore, the total time of incubation was 58 hours, the embryos being incubated without field during the first 38 hours and exposed to the PMF during the following 20 hours. At this time the eggs were

opened and the embryos double blind described.

**Preliminary experiments:**

1.- Choice of the stage at which the embryos have been exposed to the PMF:

Our purpose was to expose to the PMF embryos morphologically normal and at a stage between 8 and 10 (HH scale). For this aim it was necessary to open the eggs after a first period of incubation, for the examination of the embryos "in situ". But at the early stages of the embryonic development the opening of the shell, even during a short period, induces abnormalities. (Mann and Persaud, 1978, 1979; Fisher and Schoenwolf, 1983; Fineman et al, 1986). Therefore, experiments were performed to determine the best time for the aperture of the eggs, allowing a low frequency of abnormalities during the postincubation.

In these experiments, 140 fertil eggs were incubated, half of them during 33 hours and the other half during 38 hours. At these times, the eggs were opened and those with normal embryos were postincubated 20 additional hours. The eggs were then removed and the embryos described. The proportions of not normal embryos in the two samples were compared.

The results are indicated in Table 6. The embryos which were at stages $8^{+}$-9 at the opening of the eggs (33 hours of incubation) showed a proportion of not normal organisms reaching 54.3%. The others, which were at stages $9^{+}$-10 when the eggs had been opened presented a significantly lower proportion of not normals (17.1 % i.e three times less than the first group;

$p<0.01$). It must be recalled that all the embryos were normal at the begining of the postincubation time. This result shows that, even at developmental phases extremelly near one from the other, the sensitivity of the embryos to a change of their environmental conditions can be very different. In the present case, the abnormalities induced by the windowing of the shell affected the central nervous system, with significant differences between the two groups (Table 6).

From these experiments it was decided that embryos at stages $9^{+}$-10, will be exposed to the PMF during a 20 hour postincubation.

2.- Effect of the postincubation inside coils.

We previously observed that the incubation of the eggs, during the first 48 hours, inside no stimulated coils, does not induce an increased incidence of abnormal development in chick embryos (Ubeda et al, 1983). In the present study, eggs incubated 38 hours were opened, as indicated before (small window in the middle upper part of the shell). The embryos were examined "in situ" and the eggs reclosed. Those with embryos morphologically normal and at stages $9^{+}$-10 (a total number of 61 eggs) were returned to incubate for 20 hours. Twenty eight were postincubated inside no stimulated coils. The other 33 eggs were incubated simultaneously outside coils, in the other incubator used for the control samples (see general methodology).

The results (Table 7) indicate that the development of the embryos is not modified when the eggs, opened after a 38 hour incubation, are postincubated during 20 hours inside coils. Neither the proportion of not normal embryos nor the mean stage reached by the normal embryos at the end of the postincubation were significantly changed, when compared to the embryos postincubated outside coils.

**Results and discussion**

Ninety normal embryos, at stages $9^{+}$-10, were exposed, in vivo, to the PMF during a 20 hour postincubation (Table 8). One hundred fourteen normal embryos, at stages $9^{+}$-10, were simultaneously postincubated in vivo, but not exposed to the field (opened controls). Sixty eight eggs were also preincubated with the others, removed but not opened, and returned to incubate simultaneously to the others (non opened controls).

The embryos were double blind described at the end of the postincubation time. The results (Table 8) were the following: Comparing the proportions of not normal embryos in the opened and the non opened control groups it can be deduced that the windowing of the shell induced an increased incidence of abnormal development. Even though the difference is not significant (17.5% and 11.8% respectively; $p = 0.396$), the effect of the aperture of the eggs is under-estimated because only normal embryos, selected at 38 hours, were postincubated in the opened control group. The aperture of the shell induced malformations of the cephalic and truncal nervous systems (5% more in the opened controls).

**The effect of the PMF exposure** was different (Table 8). In the field exposed population, compared to the opened-controls, the significant increase of not normal organisms corresponds to **embryos with a malformed primitive spinal cord, the brain being normal (27.5%; 8.8% in controls; p<0.01).**

In the field exposed sample we observed 13 cases (14.3%) of extremelly thin truncal neural tube, the tissue being totally transparent and the diameter abnormally reduced (3 cases in the control group i.e 2.6%; p= 0.003). In 6 cases, the primitive spinal cord was also still opened, even though the trunk had a normal length (6.6%; 1 case in the controls i.e 0.9%; p= 0.046). These anomalies induced by the field exposure could be the results of a reduced mitotic rate, associated, in some cases, to a delay or arrest of the morphogenetic processes of the truncal nervous tissue. The histological study of the embryos reinforced this interpretation (reduced number of cells per section, non fusionned neural folds etc.)

**Therefore, the embryos are sensitive to the PMF when the exposure is started at stages 9$^{+}$-10 (38 hours incubation).** The effects, observed at the end of a 20 hour exposure, suggest that **the primitive spinal cord is the organogenetic system modified by the field exposure at these developmental stages.** In these experiments, the increased incidence of abnormal development (AR= 2.1; Table 8) was slightly higher than the incidence observed on embryos exposed to the field from the time zero of their development post laying up to 48 hours (Table 1 page 12).

**The embryonic stage of development at which the field exposure is started, more than the duration of the exposure,**

could be another "parameter" to be taken into account in a study on weak, ELF pulsed magnetic field effects on the embryonic development. The results of the present study are not inconsistent with those of A. Martin (1988). In the study of Martin, the field exposure was started at 24 hours of incubation of the fertil chicken eggs and maintained during 24 hours. At the end of their first day of incubation, the chick embryos reach stages between 6 and 7 (Hamburguer and Hamilton, 1951), which correspond to the first steps of the organogenesis of the cephalic nervous system. It seems that this developmental phase is not sensitive to the field exposure, at least for PMF parameters used in the experiments of A. Martin (also used in the Henhouse project).

It is our opinion that the response of the embryos to a weak, ELF pulsed magnetic field could be very different according, not only to the field parameters, but also to the specific developmental stage at which the field exposure is started. At least during the early development, periods as long as 24 hours include series of developmental events, extremelly different, occurring at very short time intervals. Different responses of the embryos to PMFs could be related to the short time intervals changes of the biological processes ocourring when the field exposure is started.

## TABLE 6

Effects of the opening of the eggs, at 33 hours (embryos in stages 8$^{+}$- 9) or 38 hours of incubation (embryos in stages 9$^{+}$- 10), on the further development of the embryos (without MF). The time of postincubation was 20 hours in all cases. CNS = Cephalic Nervous System; TNS = Truncal Nervous System.

| | Eggs opened at 33 hours n | % | Eggs opened at 38 hours n | % | (p) |
|---|---|---|---|---|---|
| - Total Number of Embryos | 70 | | 70 | | |
| - Embryos with anomalies of the CNS and TNS | 16 | 22.8 | 5 | 7.1 * | 0.016 |
| - Embryos with only anomalies of the CNS | 6 | 8.6 | 1 | 1.4 | 0.116 |
| - Embryos with only anomalies of the TNS | 16 | 22.8 | 6 | 8.6 * | 0.035 |
| - Total Not Normals | 38 | 54.3 | 12 | 17.1 ** | p<0.01 |

## TABLE 7

Normal and not normal embryos among fertil eggs which have been opened at time 38 hours of incubation and then postincubated 20 hours inside no stimulated coils or outside coils.

| | Postincubation inside coils | | Postincubation outside coils | | (p) |
|---|---|---|---|---|---|
| | n | % | n | % | |
| - Total Number of eggs | 28 | | 33 | | |
| - Normal embryos | 26 | 92.8 | 30 | 90.9 | NS |
| - mean stage | 15.0±0.9 | | 15.1±1.0 | | |
| - Not normal embryos | 2 | 7.1 | 3 | 9.1 | NS |
| - mean stage | 15.0±0 | | 14.2±0.7 | | |
| Abnormality ratio (% inside coils/ %outside coils) | 0.8 | | | | |

## TABLE 8

Effects of a 20 hours exposure, in vivo, to a PMF (bipolar pulse, 100 Hz repetition rate, 1.0 μT peak-to-peak amplitude, 500 μsec. duration, 85 μsec rise time), on embryos in stages 9 10 at the starting time of the exposure. All the embryos of the field-exposed group and the "opened controls" were normal at the starting time. The "non opened" controls eggs were preincubated, removed at 38 hour of incubation and returned to incubate for 20 hours more, simultaneously to the other eggs. CNS= cephalic nervous system; TNS= truncal nervous system; NOC= non opened-controls; OC= opened-controls.

| | NOC | | OC | | Exposed | | % OC / % NOC | | % Exposed / % OC | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | AR | (p) | AR | (p) |
| - Total Number of Embryos | 68 | | 114 | | 91 | | | | | |
| - Malformed for CNS and TNS | 2 | 2.9 | 9 | 7.9 | 6 | 6.6 | 2.7 | NS | 0.8 | NS |
| - Malformed for only the CNS | 0 | - | 1 | 0.9 | 0 | - | - | NS | - | NS |
| - Malformed for only the TNS | 6 | 8.8 | 10 | 8.8 | 25 | 27.5** | 1.0 | NS | 3.1 | p<0.01 |
| - Malformed for TNS and Somites | 0 | - | 0 | - | 2 | 2.2 | - | - | - | NS |
| TOTAL NOT NORMALS | 8 | 11.1 | 20 | 17.5 | 33 | 36.3** | 1.5 | NS | 2.1 | p<0.01 |
| - mean stage of normals | 14.7±1.1 | | 14.7±1.2 | | 15.1±0.7 | | | | | |
| - mean stage of not normals | 13.6±1.1 | | 14.1±1.3 | | 14.4±0.8 | | | | | |

## 3.- POSSIBLE ROLE OF THE ELECTRIC FIELD INDUCED BY A TIME-VARYING MAGNETIC FIELD

### Introduction and methods

Our aim in this study was to determine if the effects of a time-varying magnetic field on chick embryos could be dependet on the electric field (EF) induced by the MF.

For that purpose, we used a Helmholtz coil (Fig. 2). The holder of the eggs consisted of three shelves (A, B and C in the figure). In each shelf, there were ten positions (two rows of five), giving a maximum of 30 eggs incubated inside the coil, per experiment. In each experiment the eggs were placed in the coil, their long axis oriented east-west (Z axis in figure 2). The coil was located inside the incubator and a horizontal PMF, east-west oriented, was induced in the coil using a Grass SD9, 200 Hz generator. The PMF had the following characteristics: bipolar pulse with a repetition rate of 100 Hz, 500 μsec. duration, 1 μT peak-to-peak amplitude and 2 μsec. rise and fall times (field B in Table 1, page 12).

The eggs were exposed to the PMF during their first 48 hours of incubation. At the end of the 48 hours, the eggs were removed and the embryos double blind described. For each egg location in the coil it was calculated the frequency of malformed embryos i.e the proportion of malformed organisms among the fertil eggs incubated at each position in the coil.

Since the magnetic field inside the coil is almost uniform, the magnitude of the EF at the different sites of the eggs was

calculated. The EF vector induced by a time-varying magnetic field can be obtained using the Maxwell's equation:

$$E.dl = -\, d/dt \;\; B.ds \qquad (1)$$

Refering to Fig. 2 and making use of cylindrical symmetry that E has only 0 component that varies only with r, the equation (1) can be simplified as:

$$E_o .dl = -\, dB/dt.\, ds \qquad (2)$$

Which can be expressed as:

$$E_o .2\pi r = -\, dB/dt.\, \pi r^2 \qquad (3)$$

Solving for Eo and transforming to rectangular coordinates gives the x and y components of the EF.

$$E_x = 1/2\; y\; dB_z/dt$$

$$E_y = 1/2\; x\; dB_z/dt \qquad (4)$$

Since we are interested in the spatial distribution of the EF, the time derivative of $B_z$ can be supressed in the above equations to give the normalized values of the EF components.

$$E_x = 1/2\; y$$

$$E_y = 1/2\; x \qquad (5)$$

Fig .4 shows the vector of the EF in an arbitrary point P. This vector is perpendicular to the radius OP. Next, the x y

coordinates of the positions of the eggs in their support are determined. The first rows of the three shelves lie on a plane parallel to the faces of the coil, and so do the second rows. Consequently, for the purpose of calculating the EF at different sites, it is enough to considere only one row in each shelf. This will reduce the number of positions to be considered to 15. The normalized values of the EF at these positions were calculated and plotted in Fig. 5, using the equation (5).

Because the symmetry with respect to the y axis, there are 9 groups of sites, among the 15, that receive different magnitude of the EF.

## Results and discussion

A total of 285 eggs were used in 12 experiments. Table 9 shows the amplitude of the normalized EF at the 9 groups of sites where the eggs were incubated and exposed to the EMF inside the coil. The frenquency of malformed embryos among the fertil eggs incubated at each site is also indicated.

It was observed that this frequency varied significantly with the position of the eggs in the coil and found to be correlated linearly with the amplitude of the EF at these different sites ($p = 0.008$; Fig. 6). This result suggests that the EF induced by the time varying MF can intervene in the interaction of the ELF pulsed magnetic field with the embryonic development. At least for the magnetic field parameters used in this study, the lower the amplitude of the EF, the higher the frequency of malformed embryos.

This result was confirmed by two series of experiments in which field exposed embryos, located at different positions in the coil, were compared to non-exposed controls. The control embryos were incubated outside coil: In previous experiments, we observed that among 109 fertil eggs incubated inside the Helmholtz coil no stimulated (sham-exposed), the proportion of malformed organisms was 2.8% (3/109) whereas among 100 controls simultaneously incubated outside coil this frequency was 4.0% (4/100), the difference being no significant (p= 0.712).

Therefore in the following study, the controls were represented by fertil eggs incubated outside coil.

- First series of experiments: Eight experiments were performed. In each, six eggs were incubated and exposed during 48 hours to the PMF, their locations on the three shelves of the coil being those indicated in the figure 2 by the numbers 3 and 8 i.e where the mean amplitude of the EF induced by the MF is minimal (Fig. 5). A total of 47 embryos exposed to the field were compared to 90 controls. At the end of the exposure and/or incubation period, the embryos were double blind described. We observed that the proportion of malformed organisms was 14.9% (7/47) among the field exposed group and 2.2% (2/90) among the controls ($p<0.01$). Therefore the embryos exposed to the EMF, in the positions where the mean amplitude of the EF is minimal, showed a highly significant increase of strong developmental abnormalities.

- Second series of experiments: Four experiments were performed. In each, 12 eggs were incubated and exposed, during 48

hours, to the PMF, their locations on the three shelves of the coil being those indicated in figure 2 by the numbers 1, 5, 6 and 10, i.e where the mean amplitude of the EF induced by the MF is maximal (Fig. 5). Fourty six exposed eggs were compared to 56 controls. At the end of the 48 hours it was found one malformed organism among the experimental group i.e 2.2% (1/46) and 3 malformed in the control sample i.e 5.4% (3/56), the difference being no significant (p= 0.625). Therefore, the EMF exposure did not increase the frequency of malformed organisms when the relative mean value of the EF is maximal.

These experiments reinforce the result of the present study showing that the EF induced by the time varying MF can intervene in the effects of weak, ELF pulsed magnetics fields on the embryonic development.

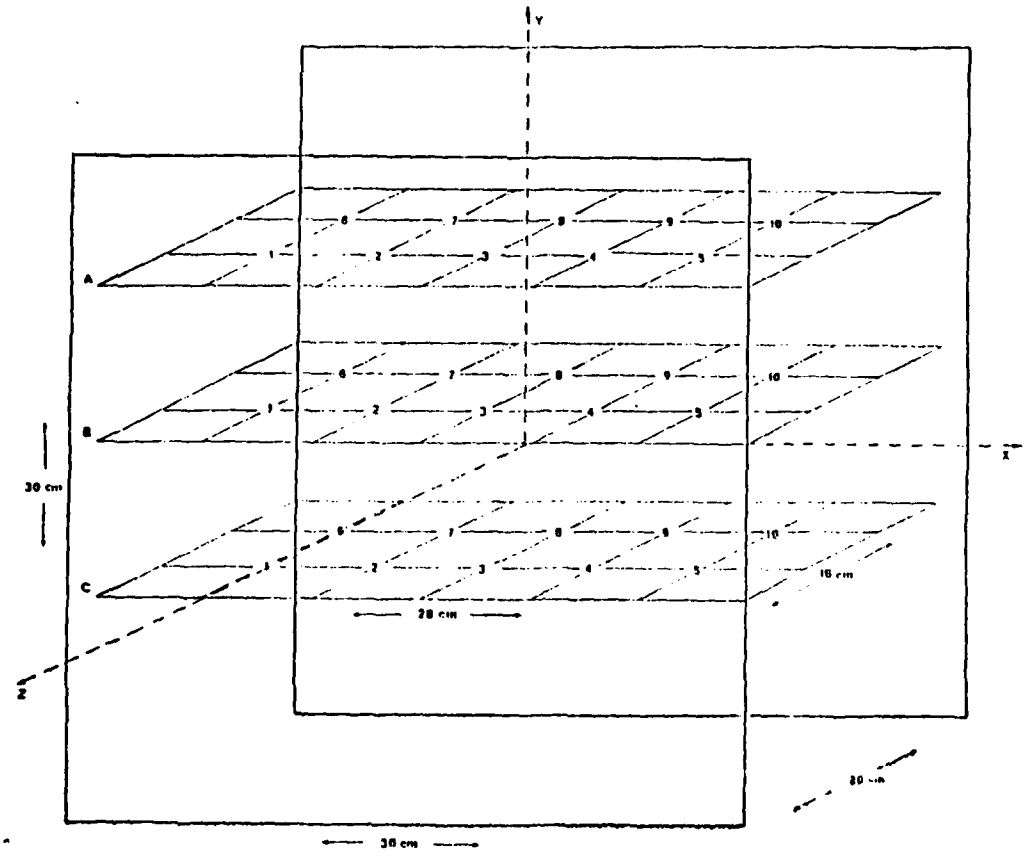


Fig. 2.- Schematic representation of the Helmholtz coil used in the study. A, B and C indicate the three shelves of the holder. Numbers from 1 to 10 indicate the sites of the eggs on each shelf.

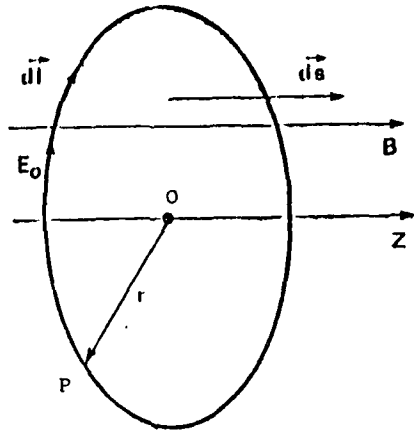


Fig. 3.- Representation of the Maxwell's equation making use of cylindrical symmetry.

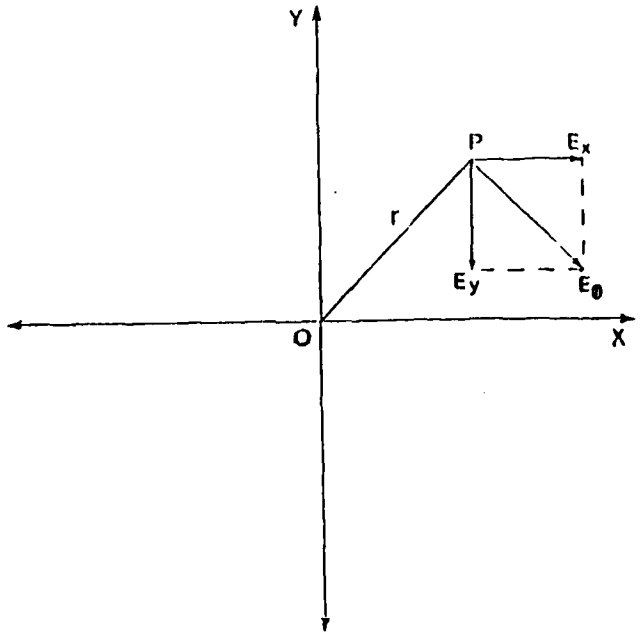


Fig. 4.- Representation of the vector of the EF ($E_O$) in an arbitrary point P.

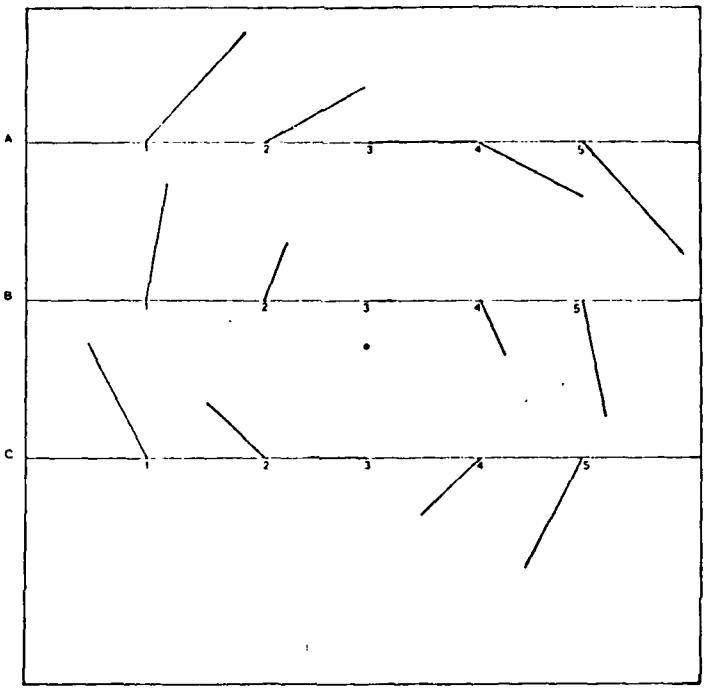


Fig. 5.- Normalized values of the EF at the positions of the eggs in the first rows.

TABLE 9

Results of a series of experiments in which fertil eggs were exposed to a EMF, induced in a Helmholtz coil (100 Hz frequency, 1 uT magnetic flux density, 500 usec duration and 2 usec rise fall times of the bipolar pulse). Exposure was performed during the first 48 hours of incubation and the embryos analized at the end of this time. The table shows the amplitude of the normalized EF at the 30 different positions of the eggs in the coil (see Fig. 2) and the corresponding frequency of malformed embryos among the fertil eggs incubated and exposed to the EMF at each site. As can be seen there are 9 groups of sites with different magnitudes of the EF.

| GROUP OF SITES | SITE IDENTIFICATION | | \|E\| | FREQUENCY OF MALFORMED EMBRYOS (%) |
|---|---|---|---|---|
| | 1st ROWS | 2nd ROWS | | |
| I | A1 = A5 | = A6 = A10 | 6.60 | 3.4 |
| II | A2 = A4 | = A7 = A9 | 5.00 | 13.2 |
| III | A3 | = A8 | 4.50 | 10.0 |
| IV | B1 = B5 | = B6 = B10 | 5.10 | 10.3 |
| V | B2 = B4 | = B7 = B9 | 2.60 | 23.1 |
| VI | B3 | = B8 | 1.00 | 19.1 |
| VII | C1 = C5 | = C6 = C10 | 5.60 | 3.2 |
| VIII | C2 = C4 | = C7 = C9 | 3.65 | 7.7 |
| IX | C3 | = C8 | 2.75 | 14.3 |

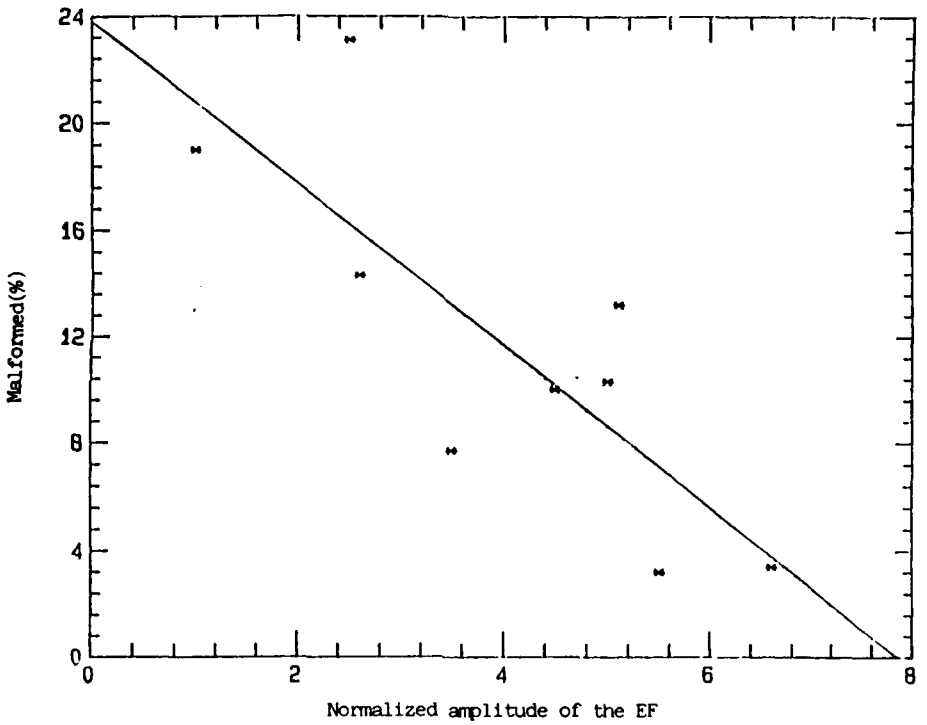


Fig. 6.- Regression of the percentage of malformed embryos on the normalized amplitude of the EF at each different site in the coil. ($p = 0.008$).

## 4.- ELECTROMAGNETIC FIELDS EFFECTS AND ORIENTATION OF THE EMBRYOS

**Introduction and Methods:**

The methods used in this study and the preliminary results can be resumed as follows: White Leghorn fertil eggs were incubated horizontally, their narrow end pointing west, without moving them, during 48 hours. At this time, the eggs were removed very carefully from the incubators and a window was opened in the upper middle part of the shell. Most of the embryos (94-97%) were found on blastoderms centrally located on the yolk. In this case, the geomagnetic orientation of their antero-posterior axis was double-blind recorded. When an egg was mishandled, the orientation of the embryo was not taken into account. As indicated in Fig. 7, the head-tail axis orientation of an embryo was North (N), South (S), East (E) or West (W) when it was strictly in one on these orientations. However, the determination can be subject to an error of approximately ±7°, due to the variations with time of the geomagnetic north direction. Any deviation from these four orientations was classified as North-West (NW), North-East (NE), South-West (SW) or South-East (SE), depending on the case.

The embryos were then taken out of the eggs and their morphology double-blind described as normal or not normal. So, the recording of the orientations and the morphological descriptions were two independent technical steps of the study.

The field exposure was started at time zero of incubation and maintained up to the end of the 48 hours. The PMF was

horizontal, east-west oriented and produced in five cylindrical coils in series using a Grass SD9, 200 Hz generator (Field A in Table 1, page 12). The pulse was bipolar (average zero), with a repetition rate of 100 Hz, duration of 500 μsec and a 85 μsec rise time. The peak-to-peak magnetic flux density was 0.4 μT, 1.0 μT or 24.9 μT depending on the experiments.

In previous experiments, we observed that most of the embryos (80-90%) were SW, S or SE oriented in the field exposed as well as in the control populations. The exposure to the PMF with a 1.0 μT peak-to-peak flux density induced a slight increase of not normal embryos (AR: 1.8; p=0.067; Table 1, page 12), distribution of the organisms in the different orientation being unchanged. The decrease of normal embryos and increase of not normals was observed among the embryos oriented to SW and SE and not among those oriented to South.

The PMF with higher flux densities had no teratogenic effect on the embryonic development but the proportion of normal embryos oriented to the South was significantly increased while it decreased in SW and SE. It was so suggested that the PMFs can also induce changes of the orientation of the embryos.

These results had to reinforced on the following questions: (1) Relation between the teratogenic effect of a PMF and the orientation of the embryos; (2) Effect of the PMFs on South oriented organisms; (3) Can the embryos change their orientation under the influence of a weak PMF?

**Results and discussion**

**- Orientation of control embryos from White Leghorn and Brown Leghorn strains.**

We compared the distribution of embryos in the different orientions for three White Leghorn and one Brown Leghorn strains. The White Leghorn strains were Shaver (total number of embryos: 649; 498 normals and 196 not normals), Hisex (total number 262; 212 normals and 50 not normals) and Dekalb (total number 246; 222 normals and 24 not normals). The Brown Leghorn strain was Carsenn (total number of embryos 107; 80 normals and 27 not normals).

We observed that the proportions of embryos found on blastoderms centrally located on the yolk and on eccentric blastoderms were significantly different in the strains (Table 10). Especially the normal embryos of these control samples with a central position on the upper part of the yolk and those with an eccentric location were found in significantly different proportions in the three White Leghorn strains and in the Brown Leghorn one. These different distributions could have consequences on the embryonic responses to a horizontal PMF: The embryos with an eccentric location on the yolk are generally found close to the air chamber of the egg and with an unegligeable inclination. As shown in Table 10, their proportion can reach approximately 15% of the total embryos in the Dekalb strain. The frequencies of normals with and eccentric location are in all the cases lower than the frequencies of not normals found on eccentric blastoderms.

We previously indicated that the tail-axis orientation of the embryos was recorded only for those found centrally located on the yolk. Fig. 8 shows the distribution of the organisms (normals plus not normals) in each orientation. In the Shaver strain the proportions of embryos oriented SW, S and SE are significantly different. This is also the case for Dekalb and Hisex embryos, except in this later strain between S and SE oriented organisms (proportions no significantly different). The Brown Leghorn embryos have a distribution more homegeneous than the White Leghorn embryos (Fig. 8), the proportions of organisms in SW, S and SE being no significantly different.

The percentages of normal and not normal embryos in each orientation (Table 11) showed that the distribution of these two types of organisms were different only for the Shaver strain in NW, W and SW directions. Even for this strain (with a higher number of embryos of the two types), the distributions of the normals and of the not normals were not different in S, SE, E, NE and N orientations.

Comparing each strain with the others (Table 12), we found that the distribution of the Dekalb normal embryos was significantly different of the two other White Leghorn. But these differences were observed, only in NW and W orientations. However, for the Brown Leghorn embryos the distributions of normal as well as not normal organisms were very different of the White Leghorn strains, except in the South, where the proportions were similar in all the cases. This similarity, for the South orientation, is not an artefact due to our criteria (compare with North, West and East oriented populations).

Therefore, the distributions of the embryos in the different orientations can be different according to the strain. The differences are particularly important between two breeds like Brown and White Leghorn. However, the proportions of the normal as well as of the not normal embryos oriented to the South were found similar in the four strains. This result could be useful for the comparison of the effects of PMFs among SW, S and SE oriented White and Brown Leghorn embryos.

- Comparison of a PMF effect on White Leghorn and Brown Leghorn embryos.

White Leghorn shaver and Brown Leghorn carsenn embryos were exposed to a PMF during 48 hours. (see general methodology). The PMF was induced in five cylindrical coils in series, as previously described (Ubeda et al, 1983; pulse A). The pulse was bipolar, with a 100 Hz repetition rate, 500 μsec duration, 0.4 μT peak-to-peak amplitude and 85 μsec rise time.

The effect of the PMF on the White Leghorn embryos was similar to the effect found in previous experiments (Ubeda et al, 1983). Fifty one exposed embryos were compared to 273 controls. Among the exposed the not normal organisms were 14 i.e 27.4% whereas they were 82 among the controls i.e 30.0% (AR=0.9; p=NS). Brown Leghorn embryos exposed in the same conditions, showed 27 not normals organisms among 40 fertil eggs i.e 67.5% while the 115 controls showed 29 not normals i.e 25.2% (AR=2.7; $p<0.01$). Therefore, a PMF that did not change the development of White Leghorn embryos, had a highly significant incidence on the

development of Brown Leghorn organisms.

We studied the orientations of 270 controls and 51 exposed White Leghorn embryos and 107 controls and 37 exposed Brown Leghorn (Table 13). The distributions of the White Leghorn embryos exposed to the field was not similar to their controls. The percentage of organisms S and SE oriented was significantly decreased in the field exposed population (Table 13). On the contrary, the proportion of those NW, W and SW oriented was significantly increased. The PMF, that had no effect on the development of these embryos, induced some changes in their orientations. Embryos moved clockwise from SE and S to SW, W and NW. The relative proportions of normals and not normals in the different orientations have not been changed by the field exposure, suggesting that normals as well as not normals had moved.

However, for the Brown Leghorn embryos exposed to the field, the percentages of organisms in each orientation or in groups of orientations were not different from their controls (Table 13, total embryos). The PMF exposure did not induce significant changes of the embryos orientations. Grouping those SW and SE oriented, we observed that in these orientations the total population was not changed by the field exposure but we found significantly less normal organisms and more not normals. This effect of the PMF was specially important in the SW orientation (Table 13, normals). On the contrary, the organisms oriented to the south did not show any increase of abnormalities.

Therefore, the Brown Leghorn embryos exposed to the field showed a significant increase of abnormal development and no

change in their orientation could be detected.

The strains White and Brown Leghorn, genetically different and with different modes of orientations, responded differently to a same PMF. The question on the lack of effect of the PMF on south oriented embryos remains unresolved.

**- Comparision of the effects of a PMF with a 1.0 μT or 24.9 μT peak-to-peak flux density on White Leghorn embryos**

The field used in the previous experiments was also used in the present study, but with a 1.0 μT flux density. This PMF had shown a slight incidence on embryos exposed 48 hours (AR=1.8; p=0.067). New experiments were performed to study the effect of the field in relation to the orientation of the embryos, and to compare the results with those obtained at 0.4 μT on White and Brown Leghorn organisms. One hundred twenty three embryos of the White Leghorn shaver strain were exposed during 48 hours and compared to 386 controls. The proportions of not normal embryos were 43.9% and 29.2% in the experimental and control samples respectively (AR=1.5; $p<0.01$). Therefore, the field induced a slight but significant increase of developmental abnormalities.

The orientation was recorded for 103 PMF exposed embryos (63 normal and 40 not normal organisms) and 321 controls (235 normals and 86 not normals). The results showed that the proportion of the population found in the different orientations was not changed by the exposure (Fig 9, A). No change was either found grouping the embryos with different orientations (Table 14, A. total embryos). Like the 0.4 μT PMF effect on Brown Leghorn

embryos, this field exposure did not induce appreciable changes in the orientation of the embryos. Once more, a decrease of normal embryos and an increase of not normals was found in SW and SE oriented organisms and not in the South ones (Fig 9, C).

These experiments were compared to others, performed in similar conditions, except that the field had a 24.9 μT pulse amplitude. Fifty nine White Leghorn shaver embryos were exposed to the field, the control group being represented by 108 organisms. The experimental sample showed 16.9% not normals and the control one 28.7% (p=0.132). Therefore this PMF had no teratogenic incidence on the organisms. The orientation of 50 exposed embryos (42 normals and 8 not normals) and 88 controls (60 normals and 28 not normals) was recorded. The results (Fig. 10; Table 14) showed that the proportions of organisms with a south orientation was significantly increased. Normal embryos had moved from SW and SE to the south. Therefore, the field induced changes of the embryos orientations and no teratogenic effect.

From these different results it is suggested that a weak PMF which has no incidence on the development can induce a part of the organisms to move and change their orientation. When a PMF induces an increased incidence of abnormal development, the embryos do not move and the teratogenic effect of the exposure is limited to organisms with specific orientations. No teratogenic effect is observed on the embryos oriented to the south.

In these series of experiments, the PMF was horizontal, east-west oriented and the electric field induced by the time-varying magnetic field had a north-south orientation. The embryos oriented to the south were parallel to the EF. Those which had

moved to the south (in the 24.9 μT PMF experiments) adopted also this orientation parallel to the EF and no teratogenic effect of the EMF could be detected. The embryos seem to act as dipoles in an EF. Therefore, experiments were performed with a PMF oriented north-south, the EF being in this case east west oriented.

**- Response of White Leghorn embryos exposed to a weak horizontal PMF oriented north-south. Preliminary results.**

In these experiments, the PMF was induced in an Helmholtz coil (represented in Fig. 2, page 32), the pulse being bipolar with a 100 Hz repetition rate, a 500 μsec duration, 1.0 μT peak-to-peak amplitude and 2 μsec rise and fall times. When applied horizontally with an east-west orientation, this field induced a significant increase of developmental abnormalities (AR=2.5; p=0.045). We also observed that the incidence of this PMF, east-west oriented, on the embryonic development, could be related to the location of the fertil eggs inside the coil. (chapter 3 of this report).

The present study had a double objective. First, to test the effect of the PMF when oriented north-south: **The eggs being located horizontally, their narrow end pointing west, as in the previous experiments, the embryos oriented to the south would be parallel to the MF and perpendicular to the east-west induced electric field. The second objective was to compare the orientation of the embryos according to the locations of the eggs inside the coil.**

In each experiment 18 White Leghorn Hisex eggs were exposed to the field. The field was horizontal, north-south oriented and the eggs located horizontally, their narrow end pointing west. In each shelf of the coil, two eggs were located near the vertical axis of the coil (axial eggs; positions n° 3 and 8 in Fig. 2, page 32) and 4 eggs on the farest locations from this axis (not axial eggs; positions n° 1, 5, 6 and 10 in Fig. 2). Twenty four eggs were simultaneously incubated but not exposed (controls). After a 48 hours exposure and/or incubation, the eggs were removed, the orientation of the embryos double blind recorded and the morphology of the organisms double blind described, as in the previous experiments.

The results were the following.

Morphological effect: Among 259 control embryos, 44 were not normals (17.0%) the malformed organisms being 14 (5.4%). Among the 193 PMF-exposed eggs (all the exposed eggs), 31 were not normal (16.1%; p=NS) with 13 malformed (6.7%; p=NS). Therefore, the global results show a lack of effect of this PMF on the embryonic development. The axial-exposed eggs and the not axial-exposed eggs were analyzed separately. In the first group we found 16 not normals among 64 embryos (25%; p=0.152) 7 being malformed (10.9%; p=0.151). In the second group the not normals were 15 among 129 embryos (11.6%; p=0.166) with 6 malformed (4.7%; p=NS). So, no significant difference with the control sample was observed, even though the axial-exposed embryos showed a higher proportion of not normals and the not axial-exposed a lower proportion than the controls. Comparing the axial-exposed to the not axial-exposed embryos, the percentages of not normal

embryos in the two groups (25% and 11.6%) were found significantly different (p=0.022). This result supports the previous ones obtained with embryos exposed to the east-west oriented PMF: The effect of the PMF seems dependent on the location of the eggs inside the coil, the axial-exposed showing a higher proportion of developmental abnormalities than the eggs in other locations. This difference, apparently EF-dependent, affects the abnormal and malformed organisms and not the non-developed. Actually the proportions of non-developed embryos were similar in the control (1.9%), the axial-exposed (4.7%; p=NS) and the not axial-exposed (2.3%; p=NS).

Orientation of the embryos: The distribution of the embryos in the different orientations showed that among all the exposed embryos, the proportion of organisms oriented to SW and SE decreased significantly (Fig 11 A), while those oriented to the south increased, even though no significantly (p=0.090). This increase of organisms in the south direction was represented by Normal embryos (13.9%; 8.3% in controls, the difference being at the limit of the significance level; p=0.058). The amount of not normals was not significantly changed neither in SW and SE nor in south.

The embryos exposed in axial positions compared to the controls, (Fig 11, B) showed a slight, no significant decrease of Normals in SW and SE (60.7%; 72.4%; p=0.086), which could be related to a slight increase of malformed organisms in these orientations (Fig. 12 D). The effect of the north-south field, if real, should be very slight. The study must be reinforced.

The population exposed to the field in not axial positions, compared to the controls (Fig. 11 A), showed a decrease of embryos SW and SE oriented, the difference being at the limit of the significance level (74.6%; 83.1%; p=0.051). This means that some embryos, a small fraction of the sample (8.5%), have moved from these orientations. It seems that they moved to the south, the increase of embryos south oriented being approximately of the same order (5%); Fig. 11 A). It was also observed that the not normals decreased significantly in SW and SE (4.0%; 10.6% in the controls; p=0.030). This decrease corresponds to abnormal organisms (Fig. 12 E) but also to malformed, so that the total of not normal embryos was significantly lower in the field exposed population. No similar effect was observed in the other orientations (even grouped). Once more, the effect of the PMF is found on SW and SE oriented embryos, as in the experiments with teratogenic east-west oriented PMFs. This result must also be reinforced.

The comparison of the axial and not axial-exposed eggs showed a significant difference of not normal embryos in the SW and SE orientations (Fig. 11 C). According to the comparisons with the control group, the axial-exposed showed in SW and SE orientations a slight increase of malformed organisms while the not axial-exposed showed a slight decrease of abnormals and malformed. This result is in accordance with the previous ones shown in the chapter 3 (Fig. 6), suggesting a role of the induced EF on the effects of the PMF exposure on the embryonic development. But no change in the proportions of normals and not normals was detected among the embryos oriented to the south. In

the same way no change was found among the embryos east and west oriented, i.e parallel to the induced electric field.

The results must be reinforced but they showed, once more, that the development of the organisms oriented to the south was not changed by the field exposure, even when the EF was perpendicular to this orientation. The different series of experiments had the same result on this peculiar orientation. The hypothesis that the geomagnetic field could have a role in the PMFs effects on embryonic development is suggested.

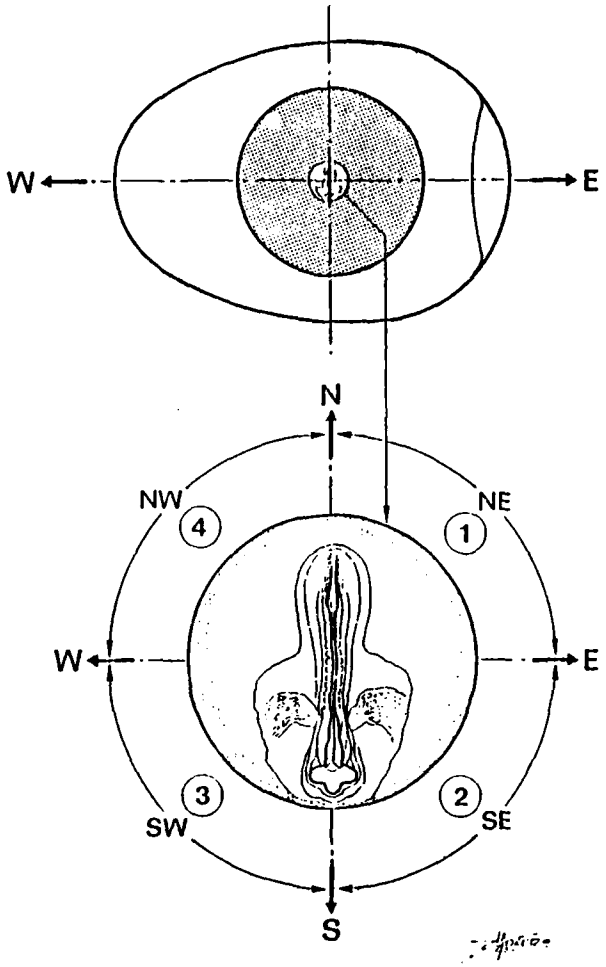


Fig. 7,- Schematic representation of an embryo oriented to the south, on a centrally located blastoderm.
The egg is placed horizontally, its narrow end pointing west. N = North; S = South; E = East; W = West; NW = North-West; NE = North-East; SE = South-East; SW = South-West.

## TABLE 10

Control Samples: Comparison of the proportions of embryos centrally located on the yolk and of those found on eccentric blastoderms in different strains. Shaver, Hisex and Dekalb are White Leghorn. Carsenn is Brown Leghorn.

| | Centrally located | | | | eccentric | | | |
|---|---|---|---|---|---|---|---|---|
| | Shaver % | Hisex % | Dekalb % | Carsenn % | Shaver % | Hisex % | Dekalb % | Carsenn % |
| **Total Embryos** | | | | | | | | |
| - Shaver (N=694) | 97.1 | | | | 2.8 | | | |
| - Hisex (N=262) | | 94.7 | | | | 5.3 | | |
| (p) | 0.078 | | | | | | | |
| - Dekalb (N=246) | | | 85.4 | | | | 14.6 | |
| (p) | p<0.01 | p<0.01 | | | | | | |
| - Carsenn (N=107) | | | | 87.9 | | | | 12.1 |
| (p) | p<0.01 | p<0.01 | | | | | | |
| **Normal Embryos** | | | | | | | | |
| - Shaver (N=498) | 99.4 | | | | 0.6 | | | |
| - Hisex (N=212) | | 95.8 | | | | 4.2 | | |
| (p) | p<0.01 | | | | | | | |
| - Dekalb (N=222) | | | 86.5 | | | | 13.5 | |
| (p) | p<0.01 | p<0.01 | | | | | | |
| - Carsenn (N=80) | | | | 97.5 | | | | 2.5 |
| (p) | p<0.01 | NS | 0.01 | | | | | |
| **Not Normal Embryos** | | | | | | | | |
| - Shaver (N=196) | 91.3 | | | | 8.6 | | | |
| - Hisex (N=50) | | 90.0 | | | | 10.0 | | |
| (p) | NS | | | | | | | |
| - Dekalb (N=24) | | | 75.0 | | | | 25.0 | |
| (p) | 0.025 | NS | | | | | | |
| - Carsenn (N=27) | | | | 59.3 | | | | 40.7 |
| (p) | p<0.01 | p<0.01 | NS | | | | | |

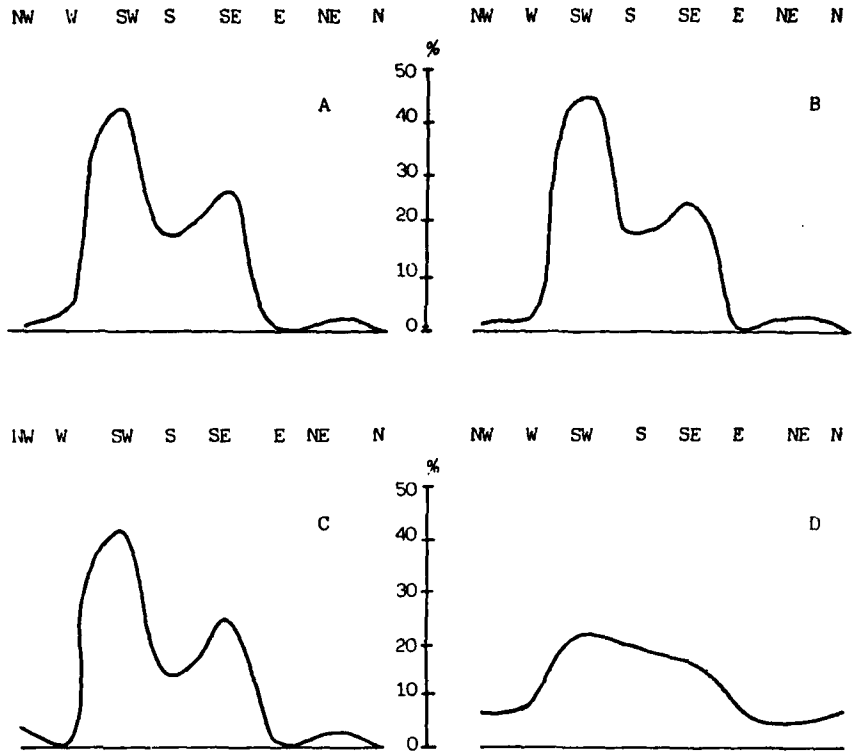


Fig. 8.- Distribution of embryos (Normals plus not normals) in the different orientations; A= White Leghorn, Shaver (N=694); B= White Leghorn, Hisex (N=262); C= White Leghorn, Dekalb (N=246); D= Brown Leghorn, Carsenn (N=107).

## TABLE 11

Comparison of the distribution of normal embryos and not normal embryos in each orientation. Only the embryos found centrally located on the yolk have been studied. Shaver, Hisex and Dekalb are White Leghorn strains. Carsenn is a Brown Leghorn strain. For each strain, the percentages (%) indicate the proportions of normal embryos in each orientation among the total number of normals or the proportions of not normals among the total number of not normals.

| | | NW | | W | | SW | | S | | SE | | E | | NE | | N | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % | n | % | n | % | n | % | n | % | n | % |
| Shaver | | | | | | | | | | | | | | | | | |
| Normals | (N=494) | 4 | 0.8 | 15 | 3.0 | 237 | 47.6 | 85 | 17.1 | 138 | 27.7 | 6 | 1.2 | 9 | 1.8 | 1 | 0.2 |
| Not Normals | (N=196) | 6 | 3.0 * | 14 | 7.1 * | 59 | 30.1 ** | 40 | 20.4 | 48 | 24.4 | 5 | 2.5 | 5 | 2.5 | 2 | 1.0 |
| | (p) | | 0.035 | | 0.020 | | p<0.01 | | NS | | NS | | NS | | NS | | NS |
| Hisex | | | | | | | | | | | | | | | | | |
| Normals | (N=212) | 0 | - | 7 | 3.3 | 94 | 44.3 | 39 | 18.4 | 56 | 26.4 | 0 | - | 5 | 2.3 | 2 | 0.9 |
| Not Normals | (N=50) | 1 | 2.0 | 0 | - | 22 | 44.0 | 12 | 24.0 | 8 | 16.0 | 0 | - | 2 | 4.0 | 0 | - |
| | (p) | | NS | | NS | | NS | | NS | | NS | | NS | | NS | | NS |
| Dekalb | | | | | | | | | | | | | | | | | |
| Normals | (N=222) | 8 | 3.6 | 1 | 0.5 | 91 | 40.9 | 30 | 13.5 | 58 | 26.1 | 0 | - | 4 | 1.8 | 0 | - |
| Not Normals | (N=24) | 1 | 4.1 | 0 | - | 11 | 45.8 | 2 | 8.3 | 2 | 8.3 | 0 | - | 1 | 4.1 | 1 | 4.1 |
| | (p) | | NS | | NS | | NS | | NS | | NS | | NS | | NS | | NS |
| Carsenn | | | | | | | | | | | | | | | | | |
| Normals | (N=80) | 6 | 7.5 | 4 | 5.0 | 19 | 23.8 | 15 | 18.8 | 16 | 20.0 | 6 | 7.5 | 5 | 6.3 | 7 | 8.8 |
| Not Normals | (N=27) | 1 | 3.7 | 4 | 14.8 | 4 | 14.8 | 5 | 18.5 | 1 | 3.7 | 1 | 3.7 | 0 | - | 0 | - |
| | (p) | | NS | | NS | | NS | | NS | | NS | | NS | | NS | | NS |

TABLE 12

**Comparison of the different strains for the orientations of the normal embryos and for the orientations of the not normal embryos (% shown in Table 11).**

| (p) | NW | W | SW | S | SE | E | NE | N |
|---|---|---|---|---|---|---|---|---|
| Normal Emorvos | | | | | | | | |
| Hisex / Shaver | NS | NS | NS | NS | NS | NS | NS | NS |
| Dekalb / Shaver | 0.011 * | 0.050 * | NS | NS | NS | NS | NS | NS |
| Dekalb / Hisex | p<0.01 ** | 0.034 * | NS | NS | NS | NS | NS | NS |
| Carsenn / Shaver | p<0.01 ** | NS | p<0.01 ** | NS | NS | p<0.01 ** | 0.03 * | p<0.01 ** |
| Carsenn / Hisex | p<0.01 ** | NS | p<0.01 ** | NS | NS | p<0.01 ** | NS | p<0.01 ** |
| Carsenn / Dekalb | NS | 0.019 * | p<0.01 ** | NS | NS | p<0.01 ** | 0.059 | p<0.01 ** |
| Not Normal Emorvos | | | | | | | | |
| Hisex/ Shaver | NS | NS | NS | NS | NS | NS | NS | NS |
| Dekalb / Shaver | NS | NS | NS | NS | NS | NS | NS | NS |
| Dekalb / Hisex | NS | NS | NS | NS | NS | NS | NS | NS |
| Carsenn / Shaver | NS | NS | NS | NS | 0.023 * | NS | NS | NS |
| Carsenn / Hisex | NS | 0.013 * | 0.012 * | NS | NS | NS | NS | NS |
| Carsenn / Dekalb | NS | NS | 0.029 * | NS | NS | NS | NS | NS |

TABLE 13

Effects of the PMF, with a 0.4 uT flux density, on the orientation of White and Brown Leghorn embryos. The percentages were calculated with respect to the total number of embryos. C = Controls; E = Field exposed; (*) = $0.05 < p < 0.1$; * = $0.01 < p \leq 0.05$; ** = $p \leq 0.01$.

| | SW | | S | | SE | | SW + SE | | S + SE | | NW+W+SW | | S+SE+E+NE | | Eccentric | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % | n | % | n | % | n | % | n | % |
| White Leghorn 0.4 uT | | | | | | | | | | | | | | | | |
| Total C=270 | 107 | 39.6(*) | 54 | 20.0 | 77 | 28.5 | 184 | 68.1 | 131 | 48.5* | 124 | 45.9* | 138 | 51.1* | 7 | 2.6 |
| Total E= 51 | 27 | 52.9 | 7 | 13.7 | 9 | 17.6 | 36 | 70.6 | 16 | 31.4 | 32 | 62.7 | 18 | 35.3 | 1 | 1.9 |
| Normals C | 86 | 31.8 | 35 | 12.9 | 58 | 21.5 | 144 | 53.3 | | | | | | | | |
| Normals E | 20 | 39.2 | 6 | 11.7 | 8 | 15.7 | 28 | 54.9 | | | | | | | | |
| Not Normals C | 21 | 7.8 | 19 | 7.0 | 19 | 7.0 | 40 | 14.8 | | | | | | | | |
| Not Normals E | 7 | 13.7 | 1 | 1.9 | 1 | 1.9 | 8 | 15.7 | | | | | | | | |
| Brown Leghorn 0.4 uT | | | | | | | | | | | | | | | | |
| Total C=107 | 23 | 21.5 | 20 | 18.7 | 17 | 15.9 | 40 | 37.4 | 37 | 34.6 | 38 | 35.5 | 49 | 45.8 | 13 | 12.1 |
| Total E= 37 | 4 | 10.8 | 5 | 13.5 | 6 | 16.2 | 10 | 27.0 | 11 | 29.7 | 11 | 29.7 | 17 | 45.9 | 7 | 18.9 |
| Normals C | 19 | 17.7 | 15 | 14.0 | 16 | 14.9 | 35 | 32.7** | | | | | | | | |
| Normals E | 0 | ** | 4 | 10.8 | 3 | 8.1 | 3 | 8.1 | | | | | | | | |
| Not Normals C | 4 | 3.7 | 5 | 4.7 | 1 | 0.9(*) | 5 | 4.7* | | | | | | | | |
| Not Normals E | 4 | 10.8 | 1 | 2.7 | 3 | 8.1 | 7 | 18.9 | | | | | | | | |

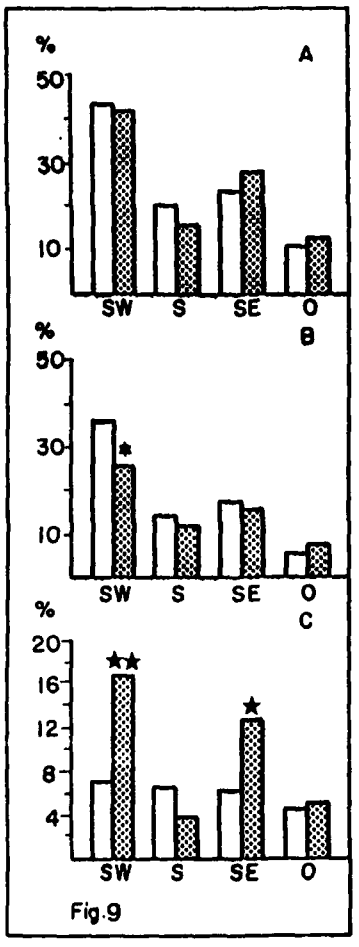


Fig.9

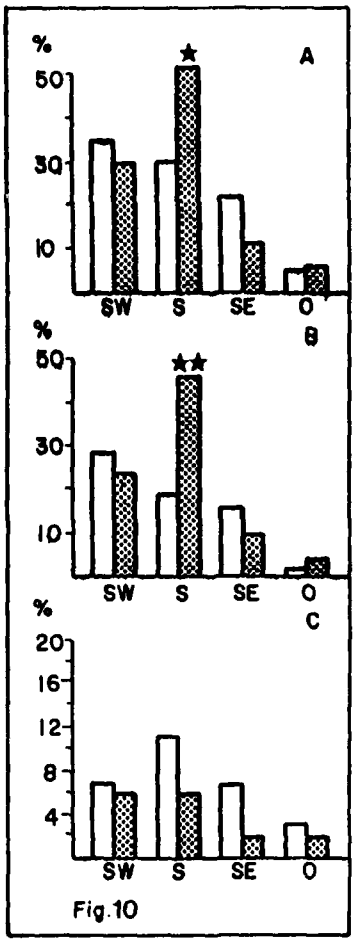


Fig.10

Fig. 9 and 10.- Orientations of embryos exposed to horizontal, East-West PMFs of 1.0 uT (Fig. 9) and 24.9 uT (Fig. 10) pulse amplitude. The percentages (%) were calculated with respect to the total number (N) of embryos in each group. Open bars: control embryos (N=321 in Fig.9; N=88 in Fig. 10); Solid bars: PMF exposed embryos (N=103 in Fig. 9; N=50 in Fig. 10). (A)= Normal and not normal embryos; (B)= Normal embryos; (C)= Not normal embryos. SW= South-West; SE= South-East; S= South; O= Orientations other than SW, S and SE. * = $0.01 < p \leq 0.05$; ** = $p \leq 0.01$.

TABLE 14

Effects of the PMF with a 1.0 μT (A) or 24.9 μT (B) flux density on the orientation of White Leghorn embryos. The percentages were calculated with respect to the total number of embryos.
C = Controls; E = Field exposed; (*) = $0.05 < p < 0.1$; * = $0.01 < p \leq 0.05$; ** = $p \leq 0.01$.

| | | SW | | S | | SE | | SW + SE | | S + SE | | NW+W+SW | | S+SE+E+NE | | Eccentric | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % | n | % | n | % | n | % | n | % | n | % |
| White Leghorn 1.0 μT | A | | | | | | | | | | | | | | | | |
| Total | C=321 | 139 | 43.3 | 65 | 20.2 | 76 | 23.6 | 215 | 67.0 | 141 | 43.9 | 160 | 49.8 | 151 | 47.0 | 8 | 2.5 |
| | E=103 | 43 | 41.7 | 16 | 15.5 | 29 | 28.1 | 72 | 69.9 | 45 | 43.7 | 52 | 50.5 | 48 | 46.6 | 2 | 1.9 |
| Normals | C | 116 | 36.1* | 44 | 13.7 | 56 | 17.4 | 172 | 53.6* | | | | | | | | |
| | E | 26 | 25.2 | 12 | 11.6 | 16 | 15.5 | 42 | 40.7 | | | | | | | | |
| Not Normals | C | 23 | 7.1** | 21 | 6.5 | 20 | 6.2* | 43 | 13.4** | | | | | | | | |
| | E | 17 | 16.5 | 4 | 3.9 | 13 | 12.6 | 30 | 29.1 | | | | | | | | |
| White Leghorn 24.9 μT | B | | | | | | | | | | | | | | | | |
| Total | C= 88 | 31 | 35.2 | 27 | 30.7* | 20 | 22.7 | 51 | 57.9(*) | 47 | 53.4 | 34 | 38.6 | 49 | 55.7 | 5 | 5.7 |
| | E= 50 | 15 | 30.0 | 26 | 52.0 | 6 | 12.0 | 21 | 42.0 | 32 | 64.0 | 15 | 30.0 | 33 | 66.0 | 0 | - |
| Normals | C | 25 | 28.4 | 17 | 19.3** | 14 | 15.9 | 39 | 44.3 | | | | | | | | |
| | E | 12 | 24.0 | 23 | 46.0 | 5 | 10.0 | 17 | 34.0 | | | | | | | | |
| Not Normals | C | 6 | 6.8 | 10 | 11.3 | 6 | 6.8 | 12 | 13.6 | | | | | | | | |
| | E | 3 | 6.0 | 3 | 6.0 | 1 | 2.0 | 4 | 8.0 | | | | | | | | |

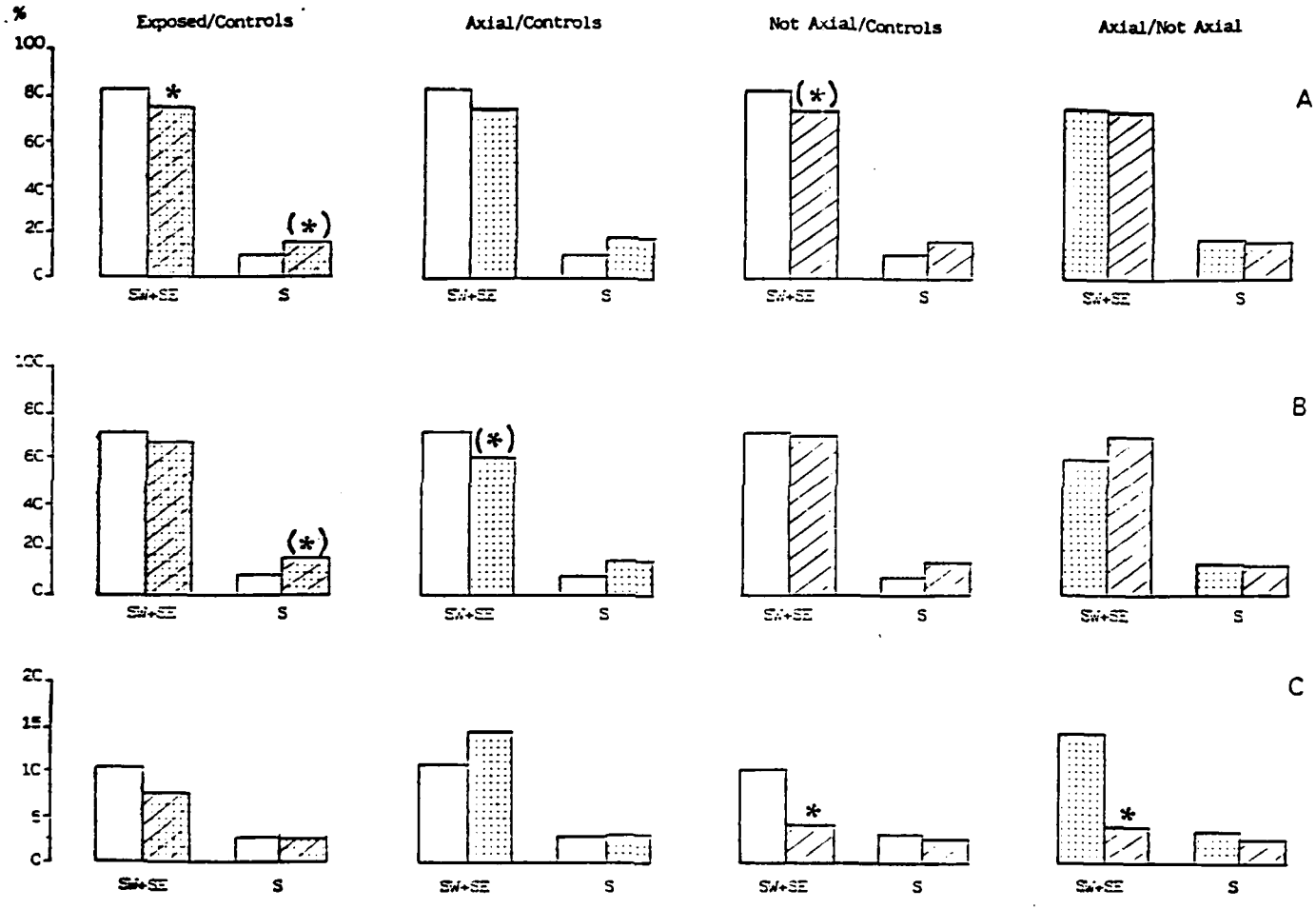


Fig. 11.- Comparison of the orientation of embryos exposed to an horizontal, North-South PMF, according to the location of the eggs inside the coil, i.e. near the vertical axis of the coil (positions nº 3 and 8, Fig. 2) or not (positions nº 1,5,6 and 10, Fig. 2). The % were calculated with respect to the total number (N) of embryos in each group. Controls (N=254); Axial locations (N=61); Not Axial locations (N=126). (A) Normal and not normal embryos; (B) Normal embryos; (C) Not normal embryos; SW + SE = Embryos oriented to South-West or South-East; S = Embryos oriented to South: ☐ = Controls; ▨ = Total exposed; ⁘ = Axial exposed;

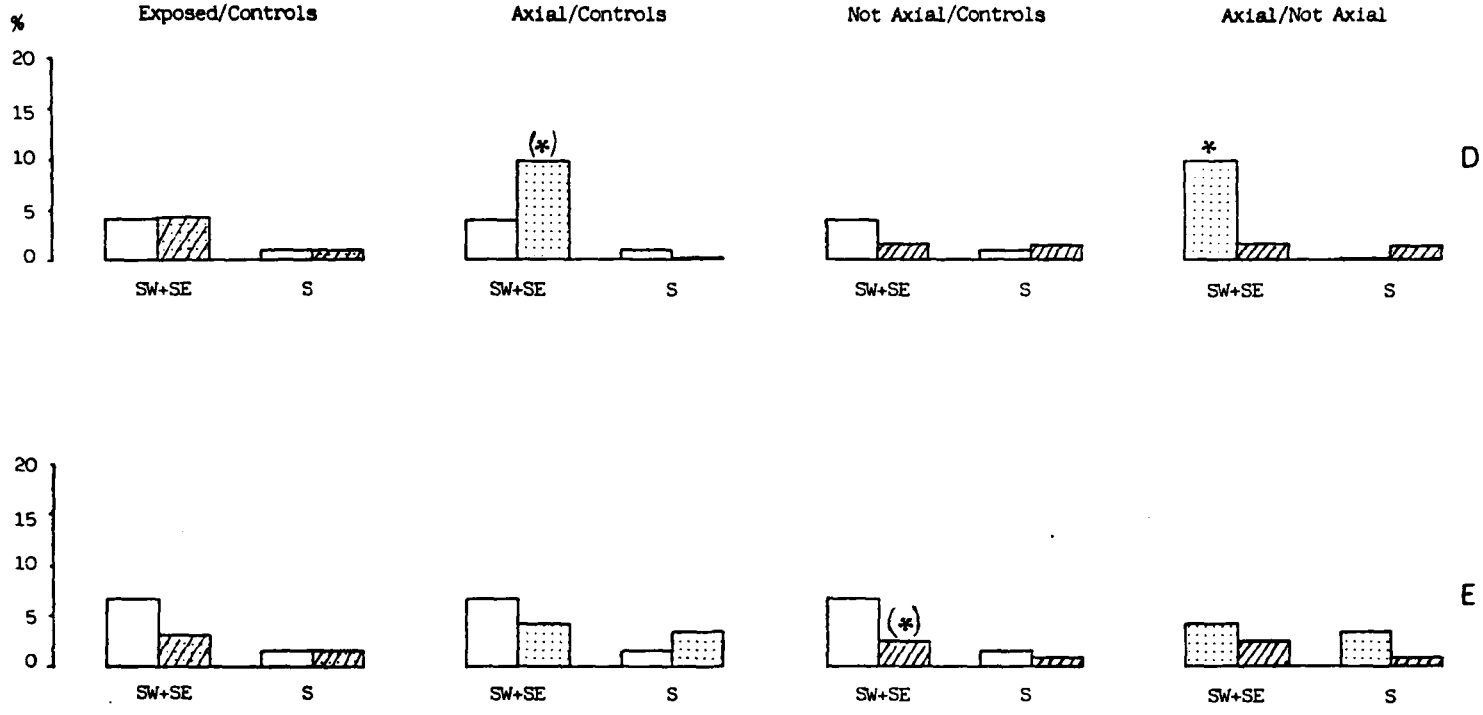


Fig. 12.- Comparison of the orientation of embryos exposed to an horizontal, North-South PMF, according to the location of the eggs inside the coil, i.e, near the vertical axis of the coil (positions nº 3 and 8, Fig. 2) or not (positions nº 1,5,6 and 10, Fig. 2). The % were calculated with respect to the total number (N) of embryos in each group. Controls (N=254); Axial locations (N=61); Not axial locations (N=126). (D) Malformed embryos; (E) Abnormal embryos; SW+SE= Embryos oriented to South-West or South-East; S= Embryos oriented to South; ☐ = Controls; ▨ = Total exposed; ⁘ = Axial exposed; ▨ = Not axial exposed; (*) = $0.05 < p < 0.1$; * = $0.01 < p < 0.05$

## 5.- PMFs EFFECTS AND EMBRYONIC DEVELOPMENT COULD BE MODULATED BY SLIGHT CHANGES OF THE LOCAL DC FIELD

### Introduction and methods

We previously observed that an experiment repeated at short time intervals, generally shows a repetitive result. This was the case of different series of experiments performed with different PMFs. In the table 1 (page 12) and the chapter 3 of this report, we indicate the result of exposures to a PMF induced in a Helmholtz coil, the pulse being bipolar with a 100 Hz repetition rate, 1 μT peak-to-peak amplitude, 500 μsec duration and 2 μsec rise and fall times: The embryos showed twice as many developmental abnormalities as its control group. This effect was repeatedly obtained in the series of experiments. Similar results were found, in the same experimental conditions, except that the magnetic flux density was 0.4 μT. (Ubeda et al, 1983).

However, we also observed that this experiment, when repeated in the same controlled conditions during a long period, can show different results.

The controlled conditions concerned the age of the hens, their food, the time interval between the laying of the eggs and their arrival at the laboratory; their conditions of storage and incubation, the PMF exposure in the same coil (20-25 eggs per experiment) located in the same incubator, the controls being outside coils, in another incubator, the same one used for all the control samples; the temperature (38 ± 0.2°C) and relative humidity (55%) in the incubators; the duration of the exposure

and incubation (48 hours); the study of the morphology of the embryos (double blind, the criteria for normality or not normality being unchanged). During each experiment, the different electrical devices of the laboratory (oscilloscope, stimulator, refrigerator etc.) were not moved, nor the metallic structures that could influence the ambient magnetic field were altered in any occasion.

For each experiment, the PMF effect was expressed by the abnormality ratio (AR; % of not normals in the field exposed group divided by the % of not normals in the control sample).

**The earth's magnetic field components** were calculated from magnetograms recorded at the nearest registration post, located at 50 Km. from our laboratory. From the magnetogram recorded during the 48 hours of each experiment, we calculated the mean values of the earth's horizontal (H) and vertical (Z) components during each experiment, as well as during its first 24 hours and second 24 hours (see page 69 the details of these calculations).

**The ambient MF of the laboratory** includes noise added to the geomagnetic field (GMF). However, these noise of different frequencies do not have any DC component (zero average). For that reason it was assumed that the variations with time of our local GMF should be similar to that of the registration post, even though the instantaneous values would be different by a constant. This constant is known to be a function of the metallic structures of the laboratory. Therefore, these structures were maintained strictly invariable. An estimation of this constant was obtained comparing measurements of the horizontal and

vertical DC fields in the laboratory (gaussmeter with an accuracy of 0.1 μT), with the H and Z values registered at the same time in the observatory. The mean values of these measurements showed 0.4 μT lower for H and 0.7 μT higher for Z in the laboratory. According to the annual horizontal and vertical isodynamic maps provided by the National Geographic Institute, the H and Z values at the geographic coordinates of our laboratory are approximately 0.5 μT lower and 0.7 μT higher than their corresponding H and Z values at the registration post. From these results, we obtained a value of the resultant F, 0.4 μT higher in the laboratory. Based on the above observations, it was decided to use the DC field values calculated directly from the magnetograms taking into account their variations and not their absolute values.

**Results and discussion**

The mean values of H, Z and the resultant F were calculated, for the first 24 hours, the second 24 hours and the 48 hours of thirteen experiments which had shown different results (ARs ranging from 0.3 to 3.5). We looked if there was a linear correlation between these H, Z, and F mean values and the results of the experiments, according to the method of the Product-moment correlation coefficient (Pearson coefficient; we assumed that the AR values had a normal distribution for each value of H, Z or F). With these coefficients and given the number of points (n=13), we looked up in the Pearson table to obtain the corresponding (p) values, considering it significant for p less

than or equal to 0.05. In case of a significant correlation, we calculated the equation of the linear fit by the least squares method (Snedecor and Cochran, 1967).

The results are shown in Table 15. The percentages of not normal embryos in the control samples presented a significant linear correlation with the mean value of H-first 24 hours. The positive correlation coefficient indicates an increase in the percentage of not normals occurring along with an increased mean value of the horizontal component. The percentages of not normals embryos in the PMF exposed samples did not show any relationship with the corresponding H values. However, the ARs which represent the relative effects of the exposures, showed strong linear correlations with the H mean values, the most significant level being observed with H-first 24 hours of the embryonic development. The AR decreases with increasing H values.

No significant relationship was found between Z values and any of the experimental data (Table 15), but the resultant F values showed significant linear correlations with the ARs. As in the case of the correlations with H mean values, the highest significance level was observed with F values of the first 24 hours of the experiments. The similarity of these results with those corresponding to the H component is not surprising due to the fact that Z showed no relationship with the PMFs effects and that F is calculated from H and Z (expression 3, page 69).

The analysis was complemented divinding the 48 hours duration of the experiments in 8 intervals of 6 hours each. It was calculated the H mean value for each interval and studied the linear fits of these values with the percentages of not normal

embryos in the control groups as well as with the ARs of the experiments. Fig. 13 shows the correlation coefficients of these linear fits vs time intervals.

The percentages of not normal embryos in control groups correlated significantly with the H mean values of the interval between 12-18 hours of the embryonic development. The ARs correlated significantly with the H values of the 8 intervals, the highest level corresponding also to the 12-18 hours interval. This period corresponds to the developmental stages 3 and 4 i.e the middle of the gastrulation phase and the definitive primitive streak.

These results suggest that slight variations of the mean value of the earth's MF horizontal component could modulate the PMFs effects on the embryonic early development. The phases of the gastrulation and the definitive primitive streak could be the most sensitive to these changes, at least during the early stages. The significant relationship found in the present study between frequencies of developmental abnormalities in control samples and H mean values suggests that some biological processes occurring at the end of the gastrulation or the special global morphology of the embryo at this stage (the only stage at which the embryo has a laminar structure) could make it sensitive to the variations of the value and/or the inclination angle of the earth's MF.

The study on embryonic development (control samples) and earth's MF was reinforced. We looked if there was a linear correlation between the percentages of not normal embryos in 31

control samples and the H values corresponding to the first 24 hours, the second 24 hours and the 48 hours of incubation of each sample. The Pearson coefficients were respectively -0.169 (NS), -0.142 (NS) and -0.158 (NS). Therefore, the linear model was not adequate in this case.

Using the method described in Abraira and Ibarz (1986), we looked for a sinusoidal correlation. The method lies to fit the dependent variable (% of not normals in each sample) to a sinusoidal wave of the independent variable (H values), looking first for the best period, in terms of the regression variance analysis.

It was found that the % of not normal embryos in the 31 control groups fit sinusoidally with H-first 24 hours, the best period being 130 nT ($p<0.01$) and the mean level of abnormalities 22.9%. A significant sinusoidal regression with H-second 24 hours was also found, with a period of 115 nT ($p<0.01$) and a 21.8% mean level of not normals. The sinusoidal fit with H-48 hours has a 115 nT period ($p<0.01$) and shows a 21.5% mean level of not normals in controls (Fig. 14).

The analysis was complemented trying to know if the % of not normals was also sinusoidally correlated with time. Actually, a significant sinusoidal correlation was found with a best period of 11 months ($p=0.03$), which represents approximately an annual variation. The same analysis done for H values and time showed significant sinusoidal relationships which best periods are 27 months ($p<0.01$), i.e approximately biannual for H-first 24 hours and H-second 24 hours. So, the variations with time of the two variables have different periods. However, the correlation of H

mean values and % of not normals in control samples could be only a relationship through the variable time.

This question was studied by a variance analysis of the percentages of not normals with two factors: H values and time (using the SAS program). For the factor time, 4 levels were considered corresponding to the seasons. For H values, 2 levels were considered. The first one corresponds to the H values for which the sinusoidal relationship suggests a high % of not normals (higher than the mean %). The second one corresponds to the H values for which the relationship predicts a low % (lower than the mean %). The analysis was done for the values of H-first 24 hours, H-second 24 hours and H-48 hours. The design of the study could be resumed as follows (example H-first 24 hours):

| class | levels | values |
|---|---|---|
| H-first 24 hours | 2 | high, low |
| Season | 4 | Winter, Spring, Summer, Autumn |

Number of observations = 31

The results of the ANOVA study are shown in Table 16. It is apparent from this analysis that the time factor is not a confounding variable of the relationship between the % of developmental abnormalities and the H-mean values. In addition, the correlation % Not Normals/H values is not season dependent.

The different parts of this study lead to a same concept: The early chick embryo could be sensitive to slight changes of the earth's MF. Variations of the horizontal component, of the

resultant F or of the inclination angle, could modify developmental processes. When a weak, horizontal PMF is added, the relationship between embryonic processes and earth's MF should be modified, not suppressed. Actually, the % of not normals in PMFs exposed samples are not correlated to the earth's MF values. But the relative effects of the time-varying fields (the ARs) do correlate with H-values. It seems that, depending on the interaction of the GMF with the embryonic processes, the AC fields would have more or less incidence on the organisms.

It is possible that only for some combinations of DC-AC fields, the AC magnetic field should be able to change some biological events in the developing organisms, as suggested by the studies of C. Blackman et al (1985), A. Liboff (1985) and Mc Leod and Liboff (1986). But, it seems that in the case of embryonic organisms, the effects of weak time-varying MFs, in relation to the DC field value, could only be estimated relatively to the control samples (for instance by the ARs), themselves being related to the DC component.

P.S.: It must be noted that the high ARs reported earlier (Ubeda et al, 1983) were encountered with H values ranging precisely where, according to the correlation of ARs with H-mean values, most abnormalities occurred in the pulsed field exposed embryos when compared to their controls.

- Earth's Magnetic Field (MF). The magnetograms recorded during the 48 hours of each of the 13 experiments were obtained from the National Geographic Intitute (NGI), Section of Geomagnetism. They represent the variations with time of the geomagnetic field (GMF) components, declination angle and temperature, recorded at the nearest registration post, located at 50 Km. from our laboratory. From each magnetogram, we calculated the mean value of the earth's horizontal component (H) during the first 24 hours (H-first 24 h.), the second 24 hours (H-second 24 h.) and the 48 hours (H-48 h.) of each experiment. An average of 48 points were taken from each graph of H variations vs. time, to calculate the corresponding H values, using the expression:

$$H = \left[ H_o + (S_H \cdot n_H) - Q_H (T - T_s) \right] \qquad 1$$

in which $H_o$ is the base value, $S_H$ the scale value, $n_H$ the distance from the base to the curve at a given point, $Q_H$ the temperature coefficient, T the temperature and $T_s$ the standard temperature of the magnetograph. H values were then plotted as a function of time and the mean values calculated from the graphs using the following expression:

$$H = \frac{1}{2T} \sum_{i=1}^{N} (t_{i+1} - t_i)(H_{i+1} + H_i) \qquad 2$$

where T is the total time, N the number of points, $t_i$ the time corresponding to the $i^{th}$ point and $H_i$ the value of H at that point.

A similar procedure was used to calculate the mean values of the earth's magnetic field vertical component (Z). From H and Z values, the resultant F was calculated according to the following expression:

$$F = \left[ H^2 + Z^2 \right]^{1/2} \qquad 3$$

TABLE 15

Significance Levels of the Linear Fits of the Experimental Data vs the Mean Values of H,Z and the Resultant F of the Earth's MF.
*: $0.01 < p \leq 0.05$; **: $0.001 < p \leq 0.01$; ***: $p < 0.001$; ARs: Abnormality ratios

| Pearson coefficients | Thirteen experiments with PMF | | |
|---|---|---|---|
| | % not normals in Controls | % not normals in PMF-exposed | ARs |
| First 24 hours | | | |
| H | 0.580* | -0.435 | -0.847*** |
| Z | -0.471 | 0.026 | 0.456 |
| F | 0.426 | -0.482 | -0.735** |
| Second 24 hours | | | |
| H | 0.460 | -0.434 | -0.662* |
| Z | -0.376 | 0.134 | 0.387 |
| F | 0.356 | -0.460 | -0.600* |
| 48 hours | | | |
| H | 0.536 | -0.444 | -0.780** |
| Z | -0.433 | 0.100 | 0.441 |
| F | 0.403 | -0.463 | -0.682** |

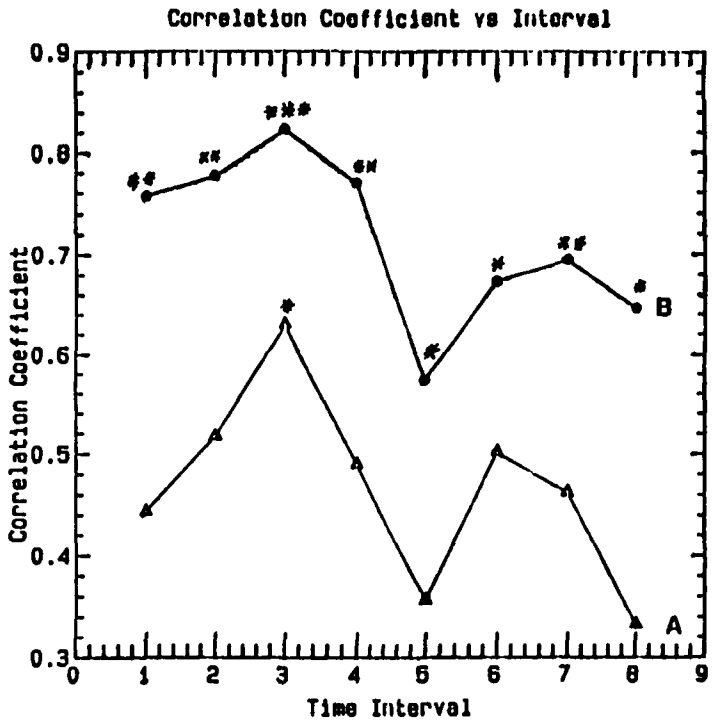


Fig. 13.- Linear Correlation Coefficients vs Time Intervals. The 48 hours of each experiment were divided into 8 intervals of 6 hours each. The H mean value of each time interval was calculated for the 13 experiments of this study. The figures show the correlation coefficients of the linear fits between these H mean values and (A) the percentages of abnormal embryos in the control samples, (B) the ARs of the experiments. * = $0.01 < p \leq 0.05$; ** = $0.001 < p \leq 0.01$; *** = $p \leq 0.001$.

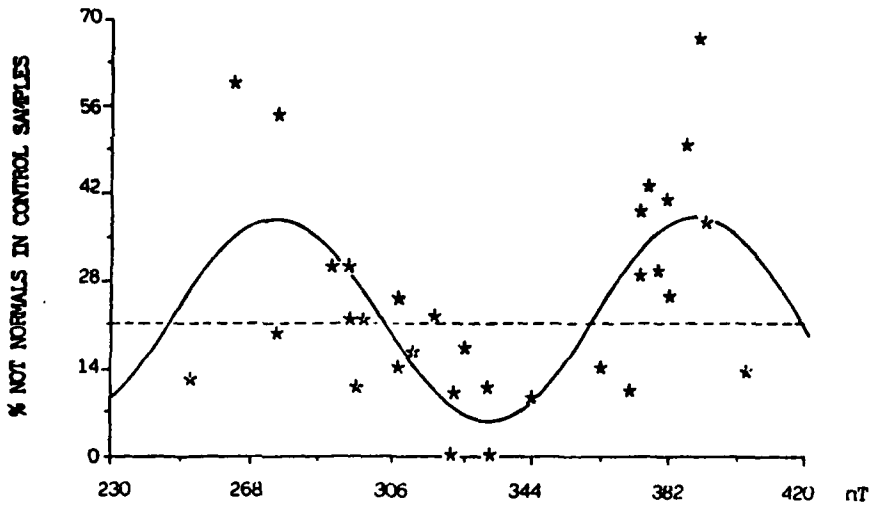


Fig. 14.- Sinusoidal correlation between the % of not normal embryos in 31 control samples and the mean values of the earth's MF horizontal component during the 48 hours incubation of each sample. Period: 115 nT; $p<0.01$; mean level of not normals: 21.5%.

**TABLE 16**

Anova study on the percentages of not normal embryos in 31 control samples, with two factors: H mean values and seasons.

| Source | DF | Sum of squares | Mean square | F value | P |
|---|---|---|---|---|---|
| H-First 24 h | 1 | 2790.818 | 2790.818 | 12.79 | p<0.01 |
| Season | 3 | 406.732 | 135.577 | 0.62 | 0.608 |
| H x Season | 3 | 304.095 | 101.365 | 0.46 | 0.709 |
| Error | 23 | 5019.943 | 218.258 | | |
| H-Second 24 h | 1 | 2393.422 | 2393.422 | 10.39 | p<0.01 |
| Season | 3 | 555.605 | 185.201 | 0.80 | 0.504 |
| H x Season | 3 | 274.934 | 91.644 | 0.40 | 0.755 |
| Error | 23 | 5297.627 | 230.331 | | |
| H-48 h | 1 | 2393.422 | 2393.422 | 10.39 | p<0.01 |
| Season | 3 | 555.605 | 185.201 | 0.80 | 0.504 |
| H x Season | 3 | 274.934 | 91.644 | 0.40 | 0.755 |
| Error | 23 | 5297.627 | 230.331 | | |

## 6.- AN HORIZONTAL, EAST-WEST ORIENTED PMF WITH A 30 Hz PULSE REPETITION RATE CAN STOP THE EARLY EMBRYONIC DEVELOPMENT.

### Introduction and Methods

Our previous results on embryos exposed to weak PMFs with a 100 Hz pulse repetition rate showed that the exposure can induce an increased incidence of developmental abnormalities. When the fields had an effect, this effect was an increase of developed organisms with morphological abnormalities.

The models proposed by A. Liboff (1985) and C. Durney et al, (1988) to explain the effects of weak time varying MFs, implicate that selected combinations of AC and DC fields produce cyclotron resonance conditions on circulating ions. The optimal conditions for an ion resonance are done when AC and DC fields are parallel, with a same magnetic flux density, the frequency of the sinusoidal AC field depending on the ionic charge (q) and mass (m), and on the DC field flux density (Bo): $= \frac{1}{2\pi} \cdot \frac{q}{m} \cdot Bo$.

The present study is the continuation of the previous ones. Its aims were (1) to approximate the conditions of exposure to those proposed by the cyclotron resonance model (A. Liboff, 1985): The field frequency was 30 Hz, calculated according to the local DC field value and the charge and mass of $Na^{+}$ and $Ca^{++}$ ions; (2) To compare the embryonic response to the previously observed when the field had a 100 Hz frequency: The AC field was a pulsed field, east-west oriented, induced in the same Helmholtz coil that in the previous studies. The pulse was bipolar, with a 1μT amplitude, 500 μsec duration and 2 μsec rise and fall

times.(3) To study, also in this case, if the EF induced by the AC field could influence the embryonic response: In each experiment, some eggs were located in axial positions (near the vertical axis of the coil; positions n° 3 and 8 in Fig. 2, page 32) and others in not axial positions (far from the vertical axis of the coil; positions n° 1,5,6 and 10 in Fig. 2).

The ambient geomagnetic field components were calculated as described in chapter 5 from the values registered at the nearest observatory and corrected by the constant differences observed between the measures done at the observatory and at the laboratory. The values were the following: Horizontal component = 25.0 μT; Vertical component = 36.4 μT; Resultant = 44.2 μT; Inclination angle = 54.5°.

The AC field frequency was 30 Hz so that it was near the resonance frequencies for $Na^{+}$ (29.5 Hz) and $Ca^{++}$ ions (33.7 Hz) according to a DC magnetic field value of 44.2 μT. The calcium and sodium pumps activities are determinant for the neural induction and calcium ions are also implicated in the processes of celular adhesion as well as optic cup formations in the chick embryo.(Barth and Barth, 1972; Spitzer, 1979; Brady and Hilfer, 1982; Shirayoshi et al,1983; Stern and Mc Kenzie, 1983).

In each experiment, 18 eggs were exposed to the PMF. Inside the Helmholtz coil, 6 eggs were located in axial positions (axial-exposed) and 12 in not axial positions (not axial-exposed). They were incubated and exposed during 48 hours and compared to 18 sham-exposed eggs located in the other incubator. The morphology of the embryos was double-blind described and the organisms classified as normal or not normal. Among the not

normals, we considered those abnormal (developed embryos with a slight anomaly or with a stage between stages 4 and 9), those malformed (developed embryos with at least one strong anomaly) and those not developed (stage lower or equal to stage 4, which is the stage of the definitive primitive streak).

**Results and discussion**

The results are shown in Table 17. Among 175 controls, 46 were not normal (26.3%), and among 175 exposed, 53 were morphologically not normal (30.3%; NS). Therefore, the PMF exposure did not significantly change the proportions of normal and not normal organisms.

However, the non-developed were significantly increased (16.0%; 6.8%; $p<0.01$) as well as the embryos with malformed optic vesicles (4.6%; none in the control group; $p<0.01$). As the global proportion of not normals was not changed, this significant increase of non-developed organisms corresponded to a decrease of not normal developed embryos i.e abnormals and malformed. It seems so that the field was effective on embryos with developmental problems which would have been developed with abnormalities in the absence of the field. The exposure stopped their development before the neurulation phase, at a stage lower or equal to stage 4.

The increase of non-developed and of malformed for the optic vesicles supports the hypothesis that the weak time-varying field modified $Na^{+}$ and/or $Ca^{++}$ transports in a fraction of the embryos. This hypothesis is reinforced by the observation that the non-

developed embryos of the field exposed group were not similar to the non-developed controls. They showed an extremely weak cellular adhesiveness, the tissues being totally disorganized during the processing of the embryos for their histological study.

The fact that the organisms sensitive to the PMF exposure were among the not normal group i.e among those with a slight (abnormals) or a strong developmental problem (malformed) suggests that individual characteristics can be determinant for the sensitivity of the organisms to a weak PMF exposure.

Fig 15 shows that among the axial-exposed embryos as well as among the not axial-exposed, the embryos with malformed optic vesicles increased (none in controls; 6.8% in axial-exposed; p=0.004 and 3.4% in not axial-exposed; p=0.024). Comparing the two groups exposed to the field, the percentages of embryos with this malformation were not significantly different. Therefore, the induction of malformed optic vesicles seems to be independent of the amplitude of the electric field inside the coil.

This is not the case of the arrest of the early development: The decrease of not normal developed embryos, i.e malformed plus abnormals, and the increase of non-developed are significant only among the embryos exposed to the PMF in not axial positions, where the relative mean amplitude of the EF is high. (Fig. 15 A and B). This result suggests that the EF also interfered with the embryonic processes in these MF exposure conditions.

For the first time, we observed an increased proportion of non-developed organisms in a population exposed to a weak PMF.

The effects are in accordance with a modification of $Na^{+}$ and/or $Ca^{++}$ pumps activities, as suggested by the cyclotron resonance model. But the incidence of the PMF exposure was restricted to a small fraction of the embryos, among those which already had developmental problems. This could be due to the fact that the experimental procedures were not those suggested by the cyclotron resonance model (the AC field was pulsed, not parallel to the DC field and with a flux density of 1.0 $\mu T$). In addition, the results suggest that, also in the present case, the EF induced by the time varying MF could have a role on the embryonic response. Compared to the same PMF but with a 100 Hz pulse repetition rate, the 30 Hz field showed very different effects. The embryos responded differently to a PMF with different frequencies, which confirmed our first studies (Delgado et al, 1982).

## TABLE 17

Effects on White Leghorn Hisex embryos of an horizontal, east-west oriented PMF. The field was induced in a Helmholtz coil (see Fig. 2). The pulse was bipolar with a 1 μT peak-to-peak amplitude, 500 μsec duration, 2 μsec rise and fall times and a 30 Hz repetition rate. The locations of the fertil eggs inside the coil were those indicated in Fig. 2 by the nΩ 1, 3, 5, 6, 8 and 10 on each shelf. Duration of the exposure and incubation: 48 h. The % indicate the proportion of each type of embryos among the total number.

| | Sham-exposed | | Field Exposed | | (p) |
|---|---|---|---|---|---|
| | n | % | n | % | |
| Total Number | 175 | | 175 | | |
| Not Normals | 46 | 26.3 | 53 | 30.3 | NS |
| Abnormals | 11 | 6.3 | 7 | 4.0 | NS |
| Malformed | 23 | 13.1 | 18 | 10.3 | NS |
| Non-Developed | 12 | 6.8 | 28 | 16.0** | p<0.01 |
| Abnormal plus Malformed | 34 | 19.4 | 25 | 14.3 | NS |
| Malformed for optic vesicles | 0 | - | 8 | 4.6** | p<0.01 |
| Non-Developed plus Malformed for optic vesicles | 12 | 6.8 | 36 | 20.6** | p<0.01 |

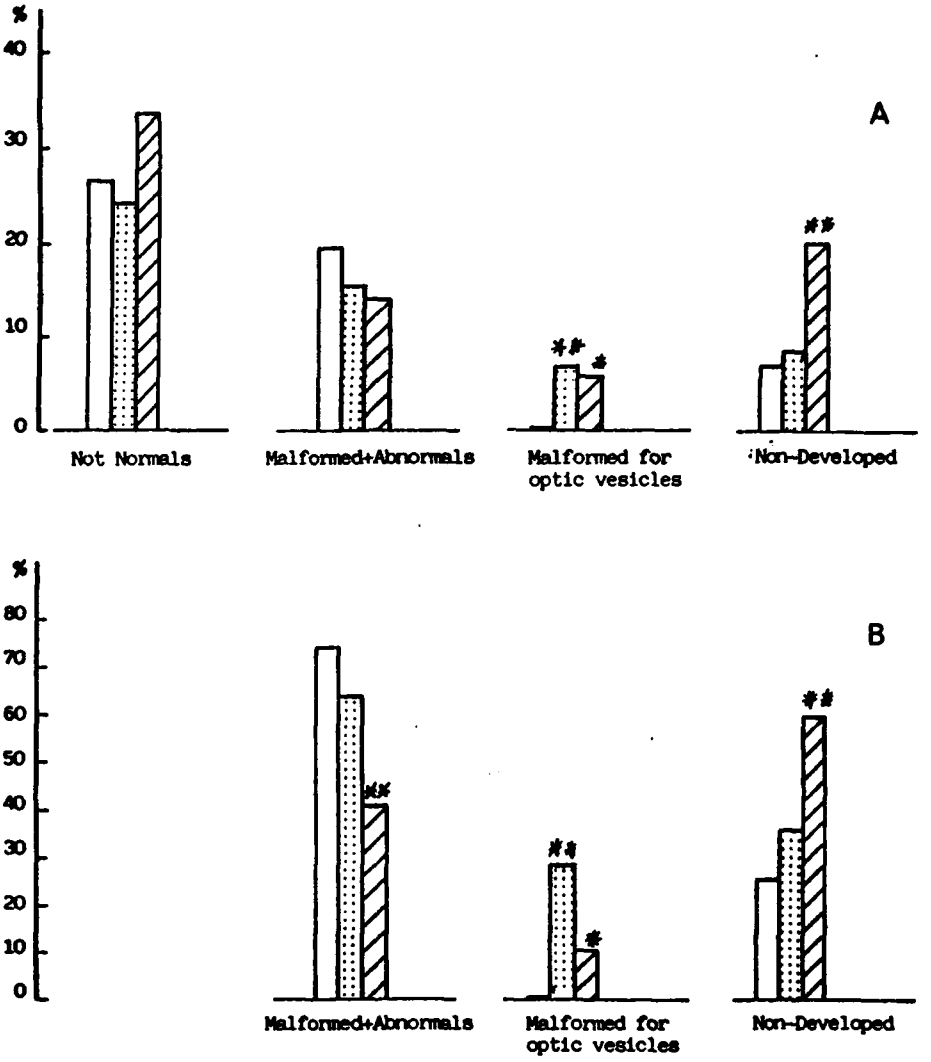


Fig. 15.- Effects of an horizontal, east-west oriented PMF on the embryonic development. Pulse: bipolar, 1.0 μT peak-to-peak amplitude, 500 μsec duration, 2 μsec rise and fall times, 30 Hz repetition rate. ☐ Sham-exposed (Total number: 175; Not normals: 46); ⁙ axial-exposed (Total number: 59; Not normals: 14); ▨ Not axial-exposed (Total number: 116; Not normals: 39). A = %/Total number of embryos for each group; B = %/Total number of Not normals for each group. * = $0.01 < p \leq 0.05$; ** = $p \leq 0.01$.

## 7.- RESPONSE OF EMBRYOS EXPOSED TO A COMBINATION OF AC AND DC MAGNETIC FIELDS, DETERMINED ACCORDING TO THE MODEL OF CYCLOTRONIC RESONANCE FOR CALCIUM IONS: PRELIMINARY STUDIES

### Introduction and Methods

In the chapter 6, we described that the embryonic response to a 30 Hz pulsed field, suggested modifications of $Na^{+}$ and/or $Ca^{++}$ transports in a fraction of the exposed population. The frequency of the AC field was determined, according to the cyclotron resonance model of A. Liboff, for a resonance of these ions in a DC ambient field with a 44.2 μT flux density. However, all the other characteristics of the AC fields were different from those theoretically necessary for a maximal resonance effect on these ions.

The cyclotron resonance model was applied to a biological system, marine diatoms, which has a clear calcium-membrane related process: movement of this ion accross the membrane controls the ability of the diatom to move. The results of the experiments show that the cells moved in response to DC and AC values of magnetic flux densities and a frequency derived from the cyclotron resonance theory to be effective on calcium ions movement across the cell membrane (Smith et al, 1987).

A developing organism is a complex biological system. It is unceasingly changing, with cells and tissues in different planes, except at the end of the gastrulation phase (i.e. approximately 18 hours of incubation), when it has a laminar organization. On this complex biological system, we attempt to study the response

to a combination of AC and DC fields which could influence calcium ions transport, according to the cyclotron resonance model.

The purpose was to expose the embryos in vivo, during their first 48 hours of development post laying, to a DC magnetic field horizontal, south-north oriented, with a 25 μT flux density and to a horizontal sinusoidal field, parallel to the DC field and with the same flux density. The frequency for calcium ions resonance was calculated to be 19 Hz (Mc Leod and Liboff, 1986).

The test apparatus consisted of three pairs of Helmholtz coils. One pair of coils with their magnetic axis along the Z axis (Fig. 16 and 17 ) controlled the vertical component of the static field (Bz) that must be brought to zero. These coils of 36-cm diameter consisted of 50 turns of 0.30 mm-diameter enamelled copper wire, the two faces of the coil being in series. Fig. 17 indicates the position of the egg holder in the coil, with its axis perpendicular to the magnetic axis (Z) of the coil. The normalized values of the vertical and radial components of the field are also indicated in Fig. 17 and 18, for each location of the eggs (10 eggs in the holder). The maximum desviation of the vertical component between the different sites of the eggs showed a maximum dispersion of 1.1 % . For the radial component, this dispersion was 2.0 %. The calibrated value of the magnetic field at the center of the coil axis was $2.40 \times 10^{2}$ μT ± 2 % /A.

Two sets of coils, with their magnetic axis along the H axis (shown in Fig. 16 and Fig. 19), i.e along the geomagnetic south-north orientation, controlled the horizontal DC component and the AC field (the two faces of each coil in series). Each pair was

placed in a Helmholtz configuration and each coil, of 34-cm diameter, consisted of 200 turns of 0.30-mm diameter enamelled copper wire. Fig. 19 shows schematically one pair of these coils with the egg holder. The normalized values of the horizontal and radial components for any of the two horizontal fields are given in Fig. 20. The maximum desviation of the aplicated horizontal component between the different sites of the eggs showed a maximum dispersion of 1.2 %. For the radial component the dispersion from one egg to other was 1.5 %. The calibrated value of the magnetic field produced by the coils, in the center of their axis was $1.07 \times 10^{3}$ $\mu$T ± 2 % / A for the DC horizontal magnetic field and $1.05 \times 10^{3}$ $\mu$T ± 2 % / A for the AC field. The calibration was done using a detector coil LDJ model SC 100 serial 119.2 cm², an integrator Walker Magnemetrics model MI-3A, an autoranging microvolt digital multimeter Keithey 197 and a digital *oscilloscope* Nicolet 3091.

The DC coils were energized by a dual DC power supply Hewlett Packard 6205.C. The AC coils were driven with a sine wave produced by a function generator Newtronics model 200 SBC. The amplitudes of the DC and AC fields were measured using a Keithley digital multimeter 130 A and observed on a Tektronix type 561 A oscilloscope.

The DC flux densities were adjusted from the values measured inside the incubator (nº 2 in Table 18) in which the coils to be stimulated (for the fields-exposed eggs) were located. Measurements of the static fields were done using a gaussmeter RFL-912 (with an accuracy of 0.1 $\mu$T). Sham-exposed eggs were

placed in an identical triple set of Helmholtz coils, located in another incubator (nº 1 in Table 18). The time-varying magnetic fields produced by the heater cycling in the incubators were measured (Table 18) using a calibrated probe with $10.5 \times 10^3 \pm 10$ $cm^2$.

The experiments were performed in a new laboratory, the incubators being also new incubators i.e different from those used in all our other studies. The incubators were placed at two meters apart and oriented to the east-west geomagnetic direction, the doors on the east side. The coils, inside each incubator, were placed so that the horizontal DC and AC fields were in the geomagnetic south-north orientation. The eggs were placed horizontally in the holder, their narrow end pointing north. Therefore, the embryos inside the eggs were perpendicular to the magnetic fields and parallel to the EF induced by the AC field. In each experiment, 10 eggs of the White Leghorn Hisex strain were exposed to the fields during a 48 hour incubation. Ten sham-exposed eggs were simultaneously incubated in the same orientation than the experimental sample and at the same temperature (37.7 ± 0.2°C) and relative humidity (60 %). At the end of the 48 hours, the eggs were removed and the embryos double blind described.

In previous experiments we studied the development of the embryos, outside coils in the two incubators. The eggs were placed horizontally, narrow end pointing north and incubated 48 hours. Ten runs were done, and we compared the % of not normal embryos as well as the mean stage of the normal organisms in the two groups. In the incubator nº 1 we found 50 not normals among

189 embryos i.e 26.5%, the mean stage of the normal embryos being 12.5±0.5. In the incubator nº 2, 53 embryos were not normal among a total of 178 embryos i.e 29.8% (p=0.479), with a mean stage of 12.4±0.8 reached by the normal organisms. In the two samples, the proportions of abnormal, malformed and non-developed embryos were also similar. Therefore, the development of the embryos during 48 hours was similar in the two incubators.

Table 18 shows the values of DC and AC fields in the incubators. The incubator nº 2 shows DC values higher and AC values lower than those measured in the incubator nº 1. For these reasons it was decided to use, for all the runs, the incubator nº 2 for the fields-exposed eggs and the incubator nº 1 for the sham eggs. Actually, the vertical and horizontal DC components were brought to 0 and 25 μT respectively for the fields-exposed eggs in the incubator nº 2, with an induced AC field of 25 μT RMS. The time-varying field produced by the heater cycling was weak when compared to the AC field induced in the coils. The sham eggs were incubated in a relatively weak DC field when compared to the experimental sample. But their ambient AC field had relatively hight values for the three components.

**Results and disccusion**

In this preliminary study, 69 embryos were exposed to the DC- AC fields during 48 hours and compared to 70 sham-exposed. The result (Table 19) was a significant increase of not normal embryos in the experimental group, with an AR of 2.6 (26.1 % in the MF-exposed, 10 % in the controls; p=0.016). This

difference was represented by abnormal embryos (20.3 %; 7.1 % in the sham sample; p=0.028). The proportions of malformed and not developed embryos were not changed by the exposure.

The development of the abnormal embryos of the fields-exposed sample was generally delayed, in comparison with the stages reached by the normal and malformed embryos of the same group or of the controls. In addition, they presented morphological abnormalities of the truncal organogenetic systems i.e the primitive spinal cord and the somites. We found nine cases of abnormal closure of the truncal nervous system (13.0 %; one case in the sham-group i.e 2.9 %; p=0.031), seven of them showing also very small somites, like unorganized small cellular masses (10.1 % of the sample; one case in the controls; p=0.033). No significant difference was found between the fields-exposed and the sham-exposed groups for the other types of abnormalities (cephalic nervous system, vascularization etc.)

These results are not exactly those expected in the case of a modification of calcium ions transport in the embryos. However, cell-cell adhesion is actually necessary to the first steps of the somites organization and the closure of the neural tube (Cheney and Lash, 1984), the calcium ions being implicated in the process of cellular adhesion. But the effect of the DC-AC fields combination was found only on the truncal systems and expressed as slight anomalies (according to our criteria and in comparison with other MFs effects).

It must be noted that the experiments described in the present study were performed in turn with others in which the AC

field flux density was 18 μT RMS instead of 25 μT. The purpose was to have a supplementary "control" of the AC-DC combination proposed by the cyclotron resonance model. Changing the AC field flux density, the exposed embryos did not show any effect of the DC-AC fields (78 exposed compared to 80 sham embryos). Therefore, even though the cyclotronic resonance conditions for calcium ions adopted in the study did not show a strong effect on the embryos, they induced an increased incidence of abnormal development.

The study will be reinforced.

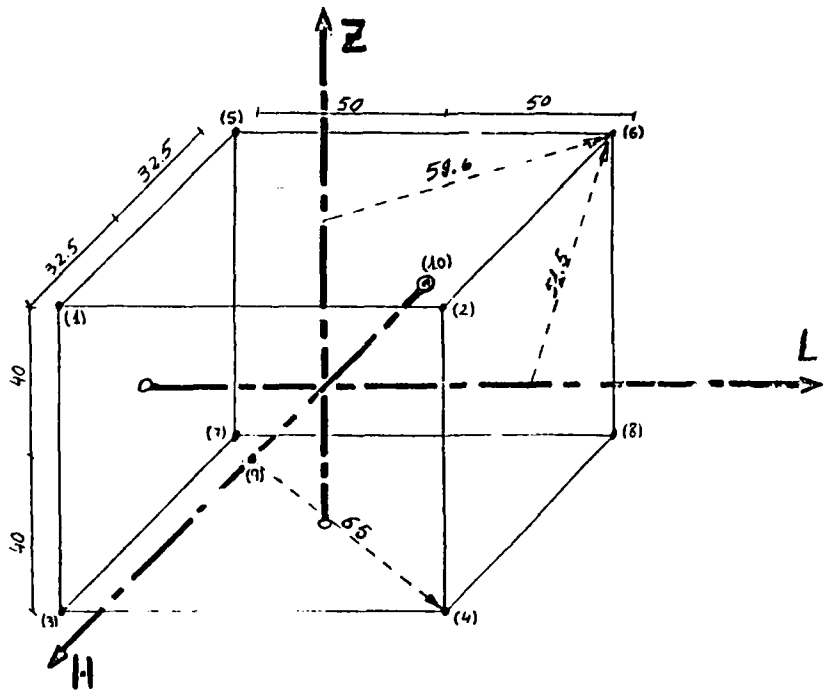


Fig. 16.- Axis of the three pairs of Helmholtz coils. H = Axis of the horizontal DC and AC fields; Z = Axis of the vertical DC component; L = Axial axis. The numbers indicate the distance (mm) between the eggs on the holder. The numbers in parenthesis indicate the sites of eggs locations.

| | z/Rz | r/Rz |
|---|---|---|
| 1 | 0.72 | 0.33 |
| 2 | " | " |
| 3 | 0.28 | " |
| 4 | " | " |
| 5 | 0.72 | " |
| 6 | " | " |
| 7 | 0.28 | " |
| 8 | " | " |
| 9 | 0.50 | 0.18 |
| 10 | " | " |

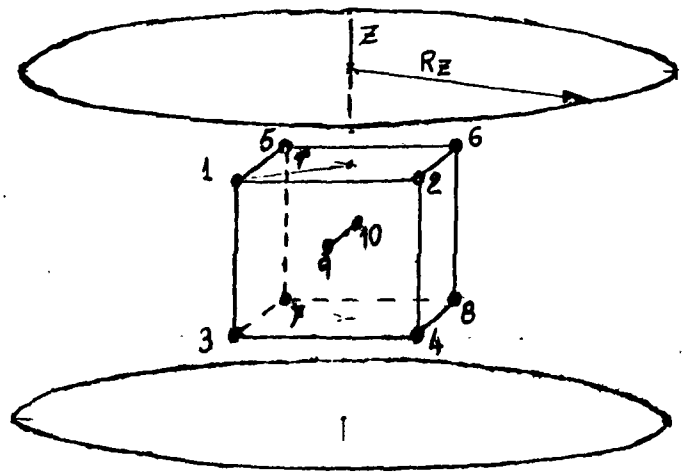


Fig. 17.- Vertical DC magnetic field: Schematic representation of the pair of coils with their magnetic axis along the Z axis, to control the vertical component of the DC field. The holder is represented with the site of each egg (numbers 1 to 10). The Table shows the normalized values (z/Rz and r/Rz) corresponding to each egg location. z and r are the components of the location vector for each egg; Rz is the radius of the coils (18 cm).

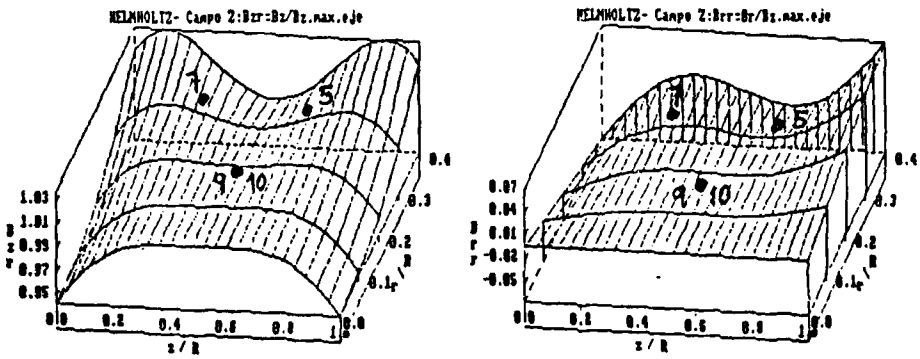


Fig. 18.- Vertical DC magnetic field: Values of the vertical Bzr and radial Brr Components, reduced to the maximum value of Bz in the axis of the coil (Fig. 17). Numbers 5,7,9 and 10 represents the locations of the eggs inside the coil with the values of the vertical and radial components at these sites.

| | h/Rh | r/Rh |
|---|---|---|
| 1 | 0.69 | 0.38 |
| 2 | " | " |
| 3 | " | " |
| 4 | " | " |
| 5 | 0.31 | " |
| 6 | " | " |
| 7 | " | " |
| 8 | " | " |
| 9 | 0.69 | 0.0 |
| 10 | 0.31 | 0.0 |

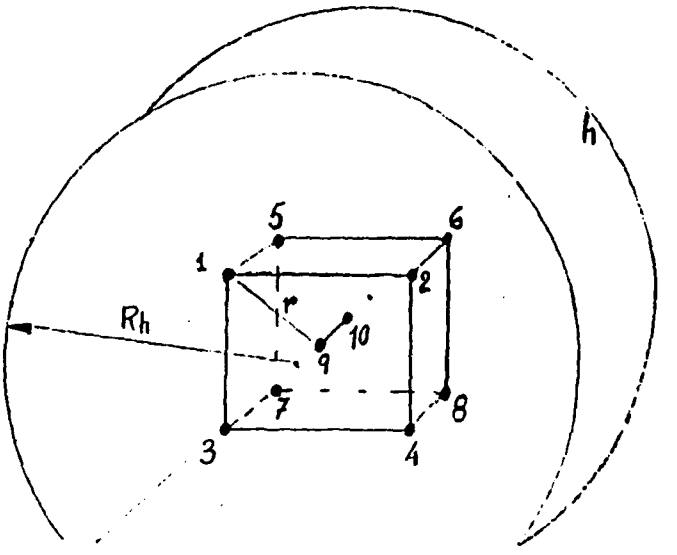


Fig. 19.- Horizontal (north-south) DC and AC fields: schematic representation of one of the two pairs of coils, their magnetic axis along the H-axis (north-south) to control the horizontal component of the static field or the AC field. The holder with 10 eggs sites is disposed so that its axis is parallel to the magnetic axis of the coils. The Table shows the normalized values (h/Rh and r/Rh) corresponding to each egg location. h and r are the components of the location vector for each egg. Rh is the radius of the coils (17 cm).

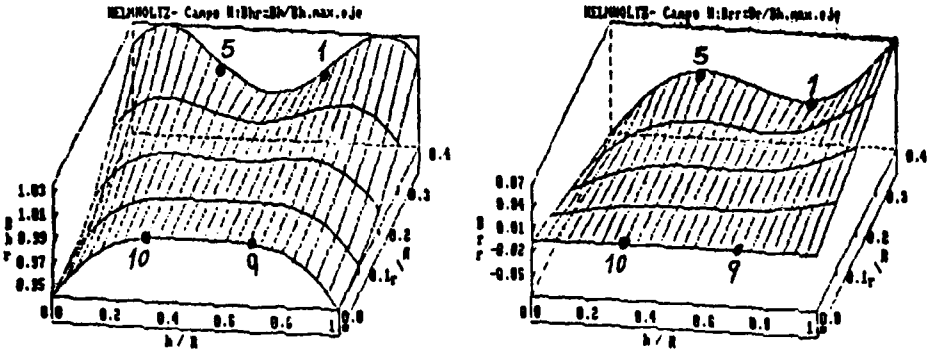


Fig. 20.- Horizontal (north-south) DC and AC fields: values of the longitudinal (Bhr) and radial (Brr) components, reduced to the maximum value of Bh in the magnetic axis of the coils. Numbers 1, 5, 9 and 10 represents the locations of the eggs inside the coils with the values of the horizontal and radial components at these sites.

## TABLE 18

Values (μT) of the vertical (Z), horizontal (M) and axial (L) components of the static magnetic fields and the AC fields (with the heater turned on) in the incubators used in this study. Negative values of Z mean downward. Positive values of M mean south to north. Negative values of L mean east to west. The measurements were done in the center of the triple set of coils inside each incubator.

| | Incubator nº 1 | Incubator nº 2 |
|---|---|---|
| Static Fields | | |
| Vertical (μT) | - 0.5 | - 16.6 |
| Horizontal (μT) | + 6.3 | + 23.6 |
| Axial (μT) | - 5.3 | - 5.4 |
| Time-Varying fields | | |
| Vertical (μT) | 12.6 | 2.0 |
| Horizontal (μT) | 16.5 | 0.3 |
| Axial (μT) | 16.6 | 6.1 |

## TABLE 19

Results of a series of experiments performed on White Leghorn Hisex embryos exposed during 48 hours to a combination of AC and DC magnetic fields. The fields were horizontal, south-north oriented, with a 25 μT flux density. The frequency of the sine wave MF was 19 Hz. * = $0.01<p\leq0.05$.

| | Sham-Exposed | MFs-Exposed | AR |
|---|---|---|---|
| - Total Number | 70 | 69 | |
| - Normals | | | |
| n | 63 | 51 * | |
| % | 90 | 73.9 * | |
| mean stage | 11.6±0.9 | 11.6±0.8 | |
| - Not Normals | | | |
| n | 7 | 18 * | |
| % | 10.0 | 26.1 * | 2.6 |
| - Abnormals | | | |
| n | 5 | 14 * | |
| % | 7.1 | 20.3 * | 2.9 |
| mean stage | 11.2±0.8 | 9.8±2.4 | |
| - Malformed | | | |
| n | 1 | 2 | |
| % | 1.4 | 2.9 | 2.1 |
| mean stage | 12 | 11.3±0.5 | |
| - Non Developed | | | |
| n | 1 | 2 | |
| % | 1.4 | 2.9 | 2.1 |

## 8.- HENHOUSE PROJECT: GENERAL CONSIDERATIONS AND "MADRID-HENHOUSE" EXPERIMENTS

### General considerations

The manuscript sent by E. Berman with his report to the ONR, presents the global aim, experiments and results of the six laboratories integrated in the project.

Each methodological procedure, data, result and interpretation, used, obtained and presented in this manuscript was discussed by the participants.

The purpose of the study was to know if a weak pulsed magnetic field can induce an increased incidence of developmental abnormalities in chick embryos, when all the experimental conditions are controlled and optimal for the embryonic development in absence of the PMF.

The purpose was also to compare the results of the different groups and put them together.For that aim, identical equipment was used in each of the six laboratories; similar conditions of storage, incubation and PMF exposure of the fertil eggs were adopted; we also tryied to have the same criteria of normality and not normality at the moment of the morphological description of the embryos.

However, the classification of non fertil eggs and fertil eggs with non-developed embryos could not be similar in the different groups. The difficulty to discriminate a fertil egg with a non developed embryo from a non fertil egg has probably induced differences in the results of the six groups. These

differences could lead to a "difficult" comparison of the proportions of fertil eggs in the six laboratories.

We also observed that, in spite of the interchange of photos with description of the embryos and discussions on this question, the determination of the embryonic developmental stage could not be done with the same criteria in every group. These differences could have been specially important on the number of somites in embryos at stages between 10 and 13. Actually, the anteriormost somite is a "rudimentary" somite which is becoming dispersed just after the stage 10. According to Hamburger and Hamilton (1951) it is not included in the counts for subsequent stages (in our group, for example, the first pair of somites is not counted, for stages higher than 10). Therefore, we think that the analysis done, comparing in the different laboratories, the stage reached by the normal embryos or their number of somite pairs should be interpreted with prudence. Because the embryos were double-blind described, the difference observed by each group between sham-exposed and PMF-exposed is available. But the criteria being different from a group to another, only the difference found by each of the six groups between sham and field-exposed samples could be compared.

Five of the six laboratories used White Leghorn fertil eggs (Hisex in our case). One group used a different breed (Arbor Acre crossed with Peterson). In experiments performed by this group with a White Leghorn strain, the proportions of not normal embryos (in sham plus exposed) were higher than 50 % in approximately half of the experiments. It was so decided that the group must change the strain used. The question is that the group

used a different breed and it was possible that, embryos from different breeds respond differently to a PMF. In fact, only in this group, the PMF-exposed sample has shown a (no significant) lower proportion of not normal embryos than the control sample. In the valid experiments (less than 50 % of not normals) performed by this group, at the same period, on a White Leghorn strain, it was found 15 not normals among 44 sham-exposed (34.1 %) and 20 not normals among 44 PMF-exposed (44.5 %; p=0.384) i.e the same trend that the other groups using White Leghorn embryos. Unfortunately this result was not indicated in the Henhouse Project manuscript.

Maybe it would be better to multiply the number of experiments done by this group on White Leghorn, up to obtain a total of 10 valid experiments comparable to the experiments performed by the other groups. The effort done to normalize all the experimental conditions was in disagreement with the decision to change the breed of eggs in one group.

## "Madrid-Henhouse" experiments

### Introduction and Methods

We received, as the other laboratories, two identical water jacketed incubators (nº 8 and 11; ONR loan) allowing to minimize temperature fluctuations. The incubators were placed at 3 meters apart, in east-west orientation, the doors at the east side. A Helmholtz coil was located inside each incubator, so that when the coil was stimulated, an horizontal east-west MF was induced. The egg holder in each coil supported 10 eggs. Measurements made at each egg position showed a maximal MF variation less than 5 % from one egg to another. The MF was pulsed. The pulse was unipolar with a 1.0 μT peak-to-peak flux density, a 500 μsec duration, a 100 Hz repetition rate and 2 μsec rise and fall times. The pulsed current was created using a 50 MHz pulse generator and the pulse shape monitored using a 20 MHz oscilloscope. The pulse generator and the oscilloscope were the same in the different laboratories (ONR loan).

The electric field induced by the MF was measured. The magnitudes of the orthogonal components of the peak EF were 0.12, 0.05 and 0.10 v/m in the axial, radial vertical and radial horizontal directions, respectively. The DC field inside each incubator was the following (incubator nº 8 and incubator nº 11, respectively): Vertical component downward, 4 μT and 22 μT; horizontal component, pointing North, 11 μT and 23 μT; Axial component, pointing West, 1 μT and 2 μT; Total field: 11.7 μT and 31.9 μT, with downward inclinations. When the heater of the

incubators were turned on, the 50 Hz RMS magnetic fields inside the incubators were the following: Vertical: 0.56 μT in the two cases; horizontal: 0.28 μT and 0.27 μT; axial: 0.047 μT and 0.064 μT; Total: 0.63 μT and 0.62 μT (measurements performed by E. Mantiply).

The temperature of incubation was maintained at 37.8 ± 0.3°C. The relative humidity was between 75 % and 83 %; the peak vertical acceleration in the coil was lower than 0.005 g. Temperature, humidity and peak acceleration for each incubator were recorded every 15 second using a six-channel multipoint recorder (ONR loan), during the 48 hours of each experiment.

In previous experiments, we observed that sham-exposed samples simultaneouly incubated in the two incubators showed very different proportions of not normal embryos. It was therefore decided to change periodically the incubator used for the field exposure. A total of 10 valid experiments were done (% not normals in sham-exposed plus field-exposed samples lower or equal to 50%). Five of these experiments were performed using the incubator nº 8 for the field-exposed group, and the other five using the incubator nº 11 (they were changed for each successive run).

The eggs were weighted before the incubation. Only those with a weight between 55 and 65 grams were kept (mean weight of the 200 eggs used: 62.6±0.15 gr).

The eggs were located horizontally, their narrow end pointing west, on the holders inside the coils, 10 eggs per holder, randomly distributed. The field exposure and/or incubation was maintained during 48 hours. The eggs were then

removed and the embryos double blind described. They were classified as normal or not normal. The not normal embryos were abnormal, malformed or non-developed (dead in the manuscript of the Henhouse Project), as previously indicated. (Chapter 6 for example). The 10 experiments were performed between April 22 and May 29, 1987.

**Results and discussion**

The results of each experiment are shown in Table 20. A total of 100 field-exposed embryos were compared to 94 sham-exposed. The proportions of not normals were repectively 31.0 % and 26.6 (p=NS). Therefore, the PMF exposure did not change the frequency of embryos morphologically not normal. This result suggests that the embryos were not sensitive to the AC field. However, the proportion of abnormal organisms, i.e with slight anomalies, was significantly decreased (3.0%; 10.6 % in the sham group; p=0.044) while the malformed increased (16.0 %; 4.2 % in the sham group; p<0.01) (Table 21; % A). The ratio of malformed embryos was 3.8, which represents a substantial effect of the field on the embryonic development. The frequency of non-developed organisms was not changed. When the not normal embryos of the two groups were compared ( Table 21; % B) it was observed that in the sham sample 40 % were abnormal and 16 % malformed, while in the field-exposed 9.7 % were abnormal (p=0.011) and 51.6 % malformed (p<0.01).

The developmental stages reached by the normal, and malformed organisms in the two groups (Table 22) were not

changed. Only the three abnormal embryos exposed to the PMF showed a higher mean stage than the abnormal controls.

Therefore, the PMF had no significant effect on the normal organisms. But it seem that the field exposure changed the development of embryos which had slight developmental problems, heightening their level of "Not normality".

These results show some similarity with those obtained on embryos exposed to a bipolar 30 Hz PMF (chapter 6): the 30 Hz MF induced an increase of non-developed organisms while abnormals and malformed decreased. These results suggested also a role of the EF in the embryonic reponse. In the present study the unipolar 100 Hz MF induced an increase of malformed organisms, while the abnormals decreased. It can not be excluded that, also in this case, the EF interfered in developmental processes.

The unipolar PMF, used in the present study, increased the proportions of malformed embryos. In practically all the cases, these embryos had a malformed primitive brain (Table 23). The field exposure induced also, but in a lower proportion, malformations of the truncal nervous system. These malformations were associated to malformed somites, heart and extraembryonary vessels (in 9 cases; none in the control; p=0.019). Therefore, the PMF induced multiple malformations in the organisms.

All the abnormal embryos of the control sample presented anomalies of the truncal nervous system, only 3 of them showing also an abnormal brain. Among the field exposed embryos, the frequency of abnormal cephalic nervous system was not changed (Table 23). But those with abnormal trunk significantly

decreased.

The different types of malformations of the central nervous system induced by the PMF are shown in Table 24: Loss of bilateral symmetry and/or disorganization of the cephalic neural tissue (10% of the exposed sample; 2.1% of the sham group; p=0.034) and not organized truncal system, the neural folds being opened, with different thickness along short neural tubes (6.0%; no case in the control group; p=0.029).

These results show that the White Leghorn Hisex embryos were sensitive to the unipolar PMF of 100 Hz frequency and 1.0 $\mu$T peak-to-peak flux density. As suggested by the global results of the Henhouse project, the PMF exposure modified the morphology of developed embryos.

The PMF induced multiple strong anomalies in organisms which would have developed only slight abnormalities in the absence of the field. The malformations observed in the PMF-exposed sample were probably irreversible and lethal. Even though the proportion of organisms sensitive to the field was small (~12%), the effect on their development was strong.

In experiments performed recently, embryos were exposed, in the conditions used in the Henhouse Project, to a PMF with a pulse repetitions rate of 30 Hz. The field exposed sample showed an increase of abnormal embryos (20%; 10.3% in the sham group) near the limit of the significance level (p=0.071). This effect of the unipolar 30 Hz field inducing abnormal embryos (slight anomalies), corresponded to a decrease of normal embryos in the population. Therefore, in this case, the incidence of the field on the embryonic development was "slight", but "normal" embryos

were sensitive to the exposure.

We observed in various studies that other weak EMFs can have strong effects on a large fraction of the exposed population, represented by normal organisms (which decreased significantly).

These different results suggest that normal, slightly-not-normal embryos and strongly alterated organisms can respond differently to weak EMFs. It is known that the embryonic development, specially at early stages, is regulated by ions transports and highly electrically charged macromolecules. Endogenous currents are created which seem to interfere in the developmental program (Weisenseel, 1983; Becker, 1984). A slight alteration or a strong anomaly in the regulating processes would probably change the endogenous currents and electric charges of cell surfaces, intracellular membranes etc. These differences could induce different sensitivities of the organisms, among a same population, to external weak EMFs.

In the case of an incidence of weak EMFs on the embryonic development, it has to be expected that embryos in different physiological states must respond to different external EMFs.

## TABLA 20

Ten experiments performed on White Leghorn Hisex embryos exposed to a PMF. The pulse was unipolar, with a 100 Hz repetition rate, 1 μT peak-to-peak amplitude, 500 μsec duration and 2 μsec rise and fall times. The field was horizontal, east-west oriented and the eggs located horizontally in the Helmholtz coil, their narrow end pointing west. The duration of the exposure and/or incubation was 48 hours for each experiment.
The percentages indicate the proportions of not normal embryos among the total number (N) of embryos in each experiment.

| Experiment | Sham-Exposed | | | Field-exposed | | | Sham-exposed plus Field-exposed | | |
|---|---|---|---|---|---|---|---|---|---|
| | | not normals | | | not normals | | | not normals | |
| nº Begining date | N | n | % | N | n | % | N | n | % |
| 1- April 22 | 9 | 2 | 22.2 | 10 | 4 | 40.0 | 19 | 6 | 31.5 |
| 2- April 25 | 10 | 3 | 30.0 | 10 | 5 | 50.0 | 20 | 8 | 40.0 |
| 3- May 2 | 9 | 3 | 33.3 | 10 | 5 | 50.0 | 19 | 8 | 42.1 |
| 4- May 5 | 8 | 4 | 50.0 | 10 | 2 | 20.0 | 18 | 6 | 33.3 |
| 5- May 9 | 10 | 4 | 40.0 | 10 | 5 | 50.0 | 20 | 9 | 45.0 |
| 6- May 12 | 10 | 2 | 20.0 | 10 | 1 | 10.0 | 20 | 3 | 15.0 |
| 7- May 16 | 10 | 5 | 50.0 | 10 | 5 | 50.0 | 20 | 10 | 50.0 |
| 8- May 20 | 9 | 0 | - | 10 | 0 | - | 19 | 0 | - |
| 9- May 23 | 10 | 2 | 20.0 | 10 | 2 | 20.0 | 20 | 4 | 20.0 |
| 10- May 27 | 9 | 0 | - | 10 | 2 | 20.0 | 19 | 2 | 10.5 |
| Total: | 94 | 25 | 26.6 | 100 | 31 | 31.0 | 194 | 56 | 28.9 |
| (p) | | | | | | 0.529 | | | |

## TABLE 21

Analysis of the not normal embryos from the sham-exposed and the PMF-exposed samples. % (A) = proportion among the total number of embryos in the sample; % (B) = proportion among the number of not normal embryos in the sample. * = $0.01<p\leq0.05$; ** = $p\leq0.01$; AR = Abnormality ratio (% Field-exposed/% sham-exposed).

| | Sham-Exposed | PMF-Exposed | AR |
|---|---|---|---|
| Total Number of Embryos (A) | 94 | 100 | |
| Number of Not Normals (B) | 25 | 31 | |
| Abnormals | | | |
| n | 10 | 3 * | |
| %(A) | 10.6 | 3.0 * | 0.3 |
| %(B) | 40.0 | 9.7 * | 0.2 |
| Malformed | | | |
| n | 4 | 16 ** | |
| %(A) | 4.2 | 16.0 ** | 3.8 |
| %(B) | 16.0 | 51.6 ** | 3.2 |
| Non-Developed | | | |
| n | 11 | 12 | |
| %(A) | 11.7 | 12.0 | 1.0 |
| %(B) | 44.0 | 38.7 | 0.9 |

**TABLE 22**

Mean stage reached by the embryos after the 48 hours exposure and/or incubation. (Results of 10 experiments). (*) Malformed whose stage could be determined.

| | Sham-Exposed | Field-Exposed |
|---|---|---|
| - Normals | | |
| n | 69 | 69 |
| mean stage | 12.5±0.7 | 12.4±0.9 |
| - Abnormals | | |
| n | 10 | 3 |
| mean stage * | 11.2±1.0 | 12.1±0.6 |
| - Malformed | | |
| n | 4 | 13 |
| mean stage | 11.4±1.8 | 11.3±1.2 |

## TABLE 23

Analysis of the abnormal and malformed organogenetic systems in the sham-exposed and the PMF-exposed samples. % (A): proportion among the total number of embryos in the sample; % (B): proportion among the not normals of the sample. In this analysis the proportion of abnormalities and/or malformations can be higher than the proportion of abnormal and/or malformed embryos (an embryo can show multiple developmental abnormalities). NS = Nervous System; * = $0.01<p\leq0.05$; ** = $p\leq0.01$; AR = Abnormality ratio.

| | Sham-Exposed<br>Total number=94(A)<br>Not normals=25(B) | PMF-Exposed<br>Total number=100(A)<br>Not normals= 31(B) | AR |
|---|---|---|---|
| **Abnormal Embryos** | | | |
| - Cephalic NS | | | |
| n | 3 | 3 | |
| %(A) | 3.2 | 3.0 | 0.9 |
| %(B) | 12.0 | 9.7 | 0.8 |
| - Truncal NS | | | |
| n | 10 | 2 | |
| %(A) | 10.6 | 2.0** | 0.2 |
| %(B) | 40.0 | 6.5** | 0.2 |
| **Malformed Embryos** | | | |
| - Cephalic NS | | | |
| n | 4 | 14 | |
| %(A) | 4.3 | 14.0* | 3.3 |
| %(B) | 16.0 | 45.2* | 2.8 |
| - Truncal NS | | | |
| n | 1 | 6 | |
| %(A) | 1.1 | 6.0 | 5.5 |
| %(B) | 4.0 | 19.4 | 4.9 |
| - Total Cephalic and Truncal NS: | | | |
| n | 5 | 20 | |
| %(A) | 5.3 | 20.0** | 3.8 |
| %(B) | 20.0 | 64.5** | 3.2 |
| - Other systems | | | |
| n | 1 | 9 | |
| %(A) | 1.1 | 9.0* | 8.2 |
| %(B) | 4.0 | 29.0* | 7.3 |

## TABLE 24

Types of malformations observed among the Sham-Exposed and PMF-Exposed samples. % (A): proportion among the total number of embryos in the sample; % (B): proportion among the not normal embryos of the sample. AR = Abnormality ratio. * = $0.01<p\leq0.05$.

| | Sham-Exposed | PMF-Exposed | AR |
|---|---|---|---|
| Total number of Embryos (A) | 94 | 100 | |
| Not Normals (B) | 25 | 31 | |
| Malformations: | | | |
| -Cephalic NS: Loss of bilateral symmetry and/or disorganization | | | |
| n | 2 | 10 | |
| %(A) | 2.1 | 10.0* | 4.8 |
| %(B) | 8.0 | 32.3* | 4.0 |
| Malformed and opened | | | |
| n | 2 | 6 | |
| %(A) | 2.1 | 6.0 | 2.9 |
| %(B) | 8.0 | 19.4 | 2.4 |
| Malformed optic vesicles | | | |
| n | 0 | 4 | |
| %(A) | - | 4.0 | - |
| %(B) | - | 12.9 | - |
| -Truncal NS: Short, opened and neural folds with different thickness | | | |
| n | 0 | 6 | |
| %(A) | - | 6.0* | - |
| %(B) | - | 19.4* | - |

## ACKNOWLEDGMENTS

We are very grateful to Dr. Thomas Rozzell. His unceasing effort to organize the Henhouse Project and to coordinate the six groups during the first period, probably the most difficult of the study, allowed us to learn many things and to participate in an exceptional research. We wish to express also our gratitude to Dr. Michael Marron for his constant support. We thank very much the EPA for the facilities offered for the entrance of the material in Spain. To the three scientific officers of our group, Drs. T. Rozzell, C. Rafferty and J. Majde, we express our immeasurable thankfulness for their kindness, support and help throughout these years of work.

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